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American Journal of Veterinary Research

VOL. II OCTOBER 1941 NO. 5

The Rôle of One Species of Cockroach and Several Species of Flies in the Dissemination of Brucella*

H. H. RUHLAND, D.V.M., and I. FOREST HUDDLESON, D.V.M., Ph.D.
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WHETHER OR NOT insects are one of the means of conveying Brucella organisms from infective material to susceptible animals and human beings has long been debated. Eyre, McNaught, Kennedy and Zammit[1] of the Mediterranean Fever Commission devoted considerable time to a study of mosquitoes and flies as vehicles of transmission of undulant fever to human beings. They were able to recover *Brucella melitensis* from the intestinal droppings of two species of mosquitoes (*Stegomyia fasciata* and *Acartomyia Zammitii*) 48 hours after feeding on the blood of infected guinea pigs. One species of the fly (*Stomoxys calcitrans*), exposed in a similar manner, showed the organism in large number in intestinal droppings 120 hours after feeding. In limited experiments these investigators failed to transmit infection to monkeys by means of either mosquitoes or flies.

Thus far in the United States insects have been given little, if any, consideration along with other agents as conveyors of infection from farm to farm or from animals to human beings. The sudden occurrence of brucellosis in one or more animals in herds which have been under close supervision and kept free from the disease for many years has puzzled many individuals. The origin of "breaks" in many noninfected herds has been a subject of considerable speculation, and thus far has not been determined.

The studies reported herein were begun with the hope of finding a lead that might help to explain why brucellosis makes its appearance in noninfected herds kept under ideal conditions.

MATERIALS AND METHODS

The common cockroach, *Periplaneta americana*, and several species of flies, *Musca domestica* L., *Muscina stabulans* F., *Stomoxys calcitrans* L., Calliphora and Lucilia, were selected for study, as they often are found in and around dairy barns. They are known to feed on the excretions of animals and, in the case of an infected placenta or fetus, could easily ingest millions of Brucella within a few minutes.

The cockroaches were exposed to infection by permitting them to feed freely for two hours on the growth of a virulent strain of *Br. abortus* on solid medium. They were then placed in a separate glass container without food. At intervals, roaches were removed, killed, dipped into ether and the intestinal contents removed and streaked over the surface of tryptose-agar plates containing crystal violet.

The flies were permitted to feed on a virulent culture in the same manner as the roaches. They were removed to a sterile glass jar in which was kept a piece of cotton soaked in sugar solution to provide food. A fresh piece of cotton was placed in the jar each day to prevent the flies from ingesting organisms that might be present in the droppings and be deposited on the cotton.

At intervals a few flies were removed from the jar and a small droplet squeezed from the anus. This was collected on a loop and streaked over the surface of a tryptose-agar plate containing crystal violet.

RESULTS AND CONCLUSIONS

The results of the two experiments are summarized in table I.

Br. abortus does not remain alive in the intestinal tract of the cockroach for more

*Journal article No. 529 (n.s.), Michigan Agricultural Experiment Station; contribution from the Section of Bacteriology.

[1]Eyre, J. W. H., McNaught, J. G., Kennedy, J. C., and Zammit, T.: Report of the Commission on Mediterranean Fever (1907), part VI, pp. 73-99.

than 24 hours. Only a few colonies were obtained on the culture medium from those that were positive. On the basis of these data, one can not conclude that the cockroach is an important conveyor of Brucella.

The data obtained on flies confirm those obtained by Eyre and associates[1] where they employed *Br. melitensis*. Although no flies were cultured later than the ninety-sixth hour after exposure, it is possible that they carry the organism for a considerable period of time. It was noted during the culturing of the droplets that the amount of growth obtained was heavier and freer from contamination 48 hours after exposure than at earlier periods.

Since conclusive data have been obtained which show that certain well-known species of flies can excrete Brucella with intestinal droplets for four days and possibly longer periods, it follows that they must be considered as one of the possible means of transmitting brucellosis from animal to animal and animal to man. Every effort should be made to prevent flies from feeding on infective materials.

TABLE I—Results of Culturing the Cockroach and Flies for Brucella Abortus

	Interval Examined After Exposure (Hrs.)	Results
Cockroaches		
11	8	5 positive, few colonies
12	12	All negative
48	24	5 positive, few colonies
18	48	All negative
15	72	All negative
6	168	All negative
Hous Flies		
2	4	2 positive, few colonies
2	16	2 positive, few colonies
2	48	2 positive, numerous colonies
2	54	2 positive, numerous colonies
2	96	2 positive, few colonies
Blow Flies		
2	24	2 positive, many colonies
2	96	2 positive, many colonies
Stable Flies		
2	4	2 positive, few colonies
2	48	2 positive, numerous colonies
2	96	2 positive, few colonies

Vitamin A Deficiencies in Ruminants*

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THE NEED OF farm ruminants for vitamin A or its precursor, carotene, for maintenance of health, vigor and reproductive capacity has become apparent through observations in the recent past.

REVIEW OF LITERATURE

Observations by feeders on feedlot cattle as far back as 1896 indicate that they were acquainted with a condition we now recognize as a vitamin A deficiency, but did not know the cause of it. Thus, Connell and Carson[1] wrote of a condition termed "fat sickness," among feeder cattle that had been on feed for some time, which was often accompanied by inflammation of the eyes or total loss of sight. They tried to reproduce this condition in feeder steers by giving them sound cottonseed meal and hulls in various proportions for 150 days continuing into hot weather. The animals selected for the experiment were 3- and 4-year-old steers. With this set-up the condition could not be produced.

The fact that Connell and Carson used cottonseed meal as a concentrate in their ration indicates that feeders in those days were of the opinion that this concentrate had something to do with the development of this condition and much was heard of "cottonseed-meal poisoning" about that time.

In the meantime much work has been done to clarify the matter. Thus, Hart and his coworkers,[2] in a long series of experiments terminating in 1924, showed vitamin A to be an essential factor in successful reproduction in cattle. Jones, Eckles and Palmer,[3] in 1926, showed that vitamin A was essential for the growth and well being of young calves and that in the absence of this factor they developed scours, became unthrifty and blind.

In 1928, Bechdel, Honeywell and Dutcher[4] studied the effect of a vitamin A-deficient ration on heifers 1 to 2½ years old. These heifers developed marked edema, loss of appetite, rapid breathing, convulsions, and blindness in one case. In two cases of pregnancy the calves were born dead.

In 1930, Halverson and Sherwood[5] published on an investigation of feeding cottonseed meal to cattle, in which they again made reference to cottonseed-meal poisoning. They were able to produce a condition that previously had been known as cottonseed-meal poisoning, but which they recognized as a vitamin A deficiency. Not only could they produce this condition by using cottonseed meal as a concentrate in the ration, but also by feeding peanut meal, linseed meal or soybean meal with a mineral mixture and a poor roughage, thus confirming the conclusion that this condition is not due to a toxic substance in cottonseed meal.

In 1933, Hart and Guilbert[6] reported the observation of a vitamin A deficiency in range cattle in California. This observation was their incentive for studying the condition more closely and soon they published observations on the storage of vitamin A in cattle,[7] to be followed a few years later by the observations on minimum vitamin A requirements with particular reference to cattle.[8]

AUTHOR'S OBSERVATIONS ON DAIRY CATTLE

The author had an opportunity to make observations of vitamin A deficiency in 1931 in five dairy cows of the Jersey breed belonging to the Division of Dairy Husbandry, Texas Agricultural Experiment Station. These cows were raised on the grounds and their feed included an abundance of green grazing in season. On December 20, 1929, when 3 to 5 years of age, they were placed on a ration consisting

*From the Division of Veterinary Science, Texas Agricultural Experiment Station; presented before the Section on Research at the seventy-eighth annual meeting of the American Veterinary Medical Association, Indianapolis, Ind., August 11-15, 1941.

[1]Connell, J. H., and Carson, J. W.: Steer feeding. Texas Agr. Exp. Sta. Bul. 41 (1896).

[2]Hart, E. B., Steenbock, H., Humphrey, G. C., and Hulse, R. S.: New observations and a reinterpretation of old observations on the nutritive value of the wheat plant. J. Biol. Chem., lxii (1924), pp. 316-322.

[3]Jones, R. I., Eckles, C. H., and Palmer, L. S.: The rôle of vitamin A in the nutrition of calves. J. Dairy Sci., ix (1926), pp. 119-139.

[4]Bechdel, S. I., Honeywell, H. E., and Dutcher, R. A.: The effect of feeding heifers a ration deficient in vitamin A. Pennsylvania Agr. Exp. Sta. Bul. 230 (1928).

[5]Halverson, J. O., and Sherwood, F. W.: Investigations in the feeding of cottonseed meal to cattle. North Carolina Agr. Exp. Sta. Tech. Bul. 39 (1930).

[6]Hart, G. H., and Guilbert, H. R.: Vitamin-A deficiency as related to reproduction in range cattle. California Agr. Exp. Sta. Bul. 560 (1933).

[7]Guilbert, H. R., and Hart, G. H.: Storage of vitamin A in cattle. J. Nutri., viii (1934), pp. 25-44.

[8]Guilbert, H. R., and Hart, G. H.: Minimum vitamin A requirements with particular reference to cattle. J. Nutri., x (1935), pp. 409-427.

exclusively of cottonseed meal and hulls. After they had been on this ration for slightly over two years, it was noticed that they would go off feed, show nasal discharge, marked swelling of the legs and other parts of the body, unsteady, painful walk, a harsh unpliable skin, convulsions, nervousness, especially when haltered and away from their accustomed surroundings, night blindness and general unthriftiness. The condition was promptly relieved by the administration of cod liver oil or green feed, but when these remedial measures were omitted the condition terminated in death. A more detailed history of the cases under observation follows:

Cow No. 41.—2-13-31: She ate part of her feed and is still milking; no other symptoms.

2-19-31: She escaped the attendant unnoticed in the darkness and grazed on green grass for about 1½ hours. She was returned to the barn and continued on the original ration. It was not until the middle of August 1932 that she was observed to show symptoms of vitamin A deficiency in the form of a swelling of the right foreleg.

8-28-32: The swelling of the right foreleg is quite marked and merges into the shoulder; slight enlargement of the right thigh. These swellings cause the cow to favor the two legs involved. There is a marked stringy discharge from both nostrils.

9-7-32: The animal is down, unable to get up. The whole of the neck now shows well-marked enlargement, merging into that of the right foreleg and shoulder. The cow was helped up and she had no difficulty in retaining a standing position. She was put on cod liver oil and made a complete recovery.

Cow No. 44.—2-13-31: This cow is still milking and cleaned up her feed. She does not as yet show definite symptoms, especially no swellings of the legs, but looks unthrifty. She has fallen off in milk production considerably during the last two weeks.

2-14-31: A marked nasal discharge.

2-15-31: Nasal discharge.

2-21-31: She shows no symptoms today and is eating her cottonseed meal and hulls.

3-9-31: Slight nasal discharge.

3-14-31: Marked nasal discharge.

3-18-31: No change.

3-25-31: Night blindness observed.

4-3-31: Getting worse. She had a convulsion this morning. She fell down on her side and struggled and rolled around violently for a short time and then got up and walked off.

4-15-31: Her allowance of cottonseed meal reduced from 8 lb. to 5 lb. because she will not eat it all.

10-2-31: The condition has gradually grown worse. She has developed swelling of the front legs during the last 60 days and today the left front leg is markedly swollen beginning at the coronet and extending and spreading into the neck immediately above the shoulders. Her right front leg is swollen but little, as is also her right hind leg. Her left hind leg is not swollen as much as her left front leg, but more than either of the right legs. There appears a marked and abruptly set off enlargement in the anterior aspect of the left tarsal joint, presumably an accumulation of synovial fluid. There is swelling around the eyes. She eats but little. She drools some and after cleaning up her small amount of feed, which she does almost regularly, there sometimes accumulates a rather large amount of liquid in the trough after she stands for a while. She shows marked night blindness, often walking around in a circle, unable to follow a straight path. She walks a little stiff.

From October 5 to December 20, 1931, the cow was fed 2½ lb. of poor-quality green sudan grass per day in addition to her cottonseed meal and hulls. This reduced all swellings and the cow recovered.

Cow No. 56.—2-13-31: The animal does not eat her cottonseed meal and hull. Temperature and respiration are normal. Unthrifty appearance is observed, manifested by somewhat thin condition for the amount of food consumed in the recent past, tucked-up abdomen, rough coat, slight watering from the eyes and a rather abundant nasal discharge. The skin is harsh, unpliable and when forced into a fold and turned loose, only slowly smoothens out again.

2-15-31. The front legs above the carpal joint are beginning to swell.

2-18-31: The cow fell down this morning on the way to the barn. She is reported to have rolled completely over her back three times before regaining her feet. Her right front leg is markedly swollen, especially above the carpal joint, but also noticeably below and extending to the foot. Her left leg is swollen but slightly, while nothing can be detected on her hind legs.

2-21-31: There is not much change. The cow refuses her feed.

2-23-31: She ate a little of her cottonseed meal today. Her body temperature is 100.4 F., and she now also shows a swelling of the hind legs beginning at the hock and merging into the thigh.

2-25-31: A marked swelling of both front legs is observed, and also of the hind legs. Beginning today she is put on two daily doses of 125 cc. of cod liver oil.

2-28-31: No improvement is shown. She ate

but little of her evening feed and looks gaunt. She seems to walk a little better.

3-2-31: She cleaned up her feed this morning and also in the evening. She is walking much better, but there is no noticeable decrease in the swellings.

3-3-31: The cow is walking much better, but she has a profuse nasal discharge. She refused part of her morning feed and nibbled at it in the evening.

3-5-31: The nasal discharge is still quite profuse. The swelling in the hind legs has disappeared, but no improvement can be noted in the swelling in the front legs. She is cleaning up about one half of her feed.

3-9-31. There is a fairly large amount of nasal discharge, but otherwise the cow seems to be improving gradually. The swelling in the front legs is going down and her feed consumption has been so satisfactory during the last few days that her cottonseed-meal allowance has been increased from 2 to 3 lb.

3-14-31: The cow ate her 3 lb. of cottonseed meal for a couple of days, but yesterday morning went off feed entirely and has not eaten any meal since. She looks and acts much brighter than she has for a long time. The discharge from her nose is quite profuse, but this seems to vary from day to day. The swelling in her front legs is not entirely gone yet, although there is but little left.

The cow eventually recovered on the cod liver oil treatment with no change in feed.

Cow No. 198.—2-13-31: The cow is off feed. She does not show any swelling of the legs, but looks unthrifty.

2-14-31: Now she shows a marked swelling of the legs between the carpal and elbow joints, but no swelling of the hind legs. There is a marked nasal discharge.

2-18-31: The swelling of the front legs is increasing and has already involved the shoulder. The hind legs do not as yet show swelling. She walks stiff in front, as though in pain, and shows a stringy nasal discharge (fig. 1).

2-21-31: The cow seems worse today, but still shows no swelling of the hind legs. She refused her feed and has a marked nasal discharge.

2-23-31: There is no change. The body temperature is 102.2 F.

2-25-31: While being driven into the barn the cow fell down and was unable to get up. Several attempts were made to help her up, but all were unsuccessful. She was then sacrificed for autopsy.

Autopsy showed a gelatinous infiltration in the affected regions, namely, the front legs above the carpal joints, the neck, along the spinal column on either side of the spinous processes and as far back as the lumbar region, and in the superior angle of the thoracic aperature. The thoracic cavity contained about 150 cc. of a clear, yellow fluid in each half, and the pericardial sac about 75 cc. of the same fluid. The spleen was not as firm and inflexible as that of No. 307, but was more so than normally. The liver was pale, firm and contained numerous small, light areas throughout. The eyes were normal. No other lesions were found.

Cow No. 307.—2-13-31: The animal is completely off feed, has a normal temperature and rate of respiration and an unthrifty appearance manifested by a somewhat thin condition for the amount of food consumed in the recent past. She has a tucked-up abdomen, rough coat, watering from the eyes and an abundant nasal discharge. She shows a marked swelling of all legs. The hind legs are swollen just above the hock joints, while in the front legs the swelling extends up to the point of the shoulders. She shows a stiff, painful walk. The skin is harsh, unpliable and when forced into a fold and turned loose only slowly smoothens out again.

Fig. 1. Cow 198, showing nasal discharge and swelling of front legs.

2-14-31: The swelling of the legs is more marked, especially the hind legs, in which it has not only increased in circumference, but also now extends farther up the legs.

2-15-31: The swelling of the front legs has now reached the neck. There is a discharge from the nose and eyes.

2-18-31: The cow fell down last night on her way to the barn, and although the attendants tried to get her up, they could not. She remained down for about five hours. The swelling of the hind legs, the front legs and shoulders, half way to the withers, is marked, while the swelling in her neck, although noticeable, is not as marked as the swelling in the legs. In walking she sways from side to side in front.

2-21-31: She walks with difficulty and pants fast while standing in the barn. She is refusing her feed and shows a marked nasal dis-

charge. The eyes are watering slightly. The swellings seem to be increasing.

2-23-31: The neck is swollen so badly that it would hardly go into the stanchion. The swelling extends from immediately back of the ears to the shoulders and from 8 in. below the withers to the hoofs in front. In the hind legs the swelling begins at the hoof and merges into the thighs. The body temperature is 100.4

Fig. 2. Cow 307, photographed two hours before she was destroyed.

F. She breathes normally and is off feed.

2-25-31: The cow fell down while being driven into the barn and was unable to get up (fig. 2). Several attempts were made to help her up, but all were to no avail. She was sacrificed for autopsy.

At autopsy the subcutaneous tissue was dry and the hide difficult to remove. The subfascial and intermuscular connective tissues were infiltrated with a clear, gelatinous substance from which liquid could be expressed upon pressure. In the region of the neck the greatest amount of this gelatinous substance had accumulated next to the ligamentum nuchae. In some places of the affected parts the muscles showed rather moist on section. The thoracic cavity and pericardial sac contained an excess of a clear, yellow fluid. The left ventricle showed numerous subendocardial hemorrhages. The lungs revealed nothing of note. The liver was pale and firmer than normal, and the edges round. The spleen was unusually firm, dry on section and but little flexible. The subdural space contained a large amount of fluid. The eyes were normal. No other lesions were found.

OBSERVATIONS ON STEERS

Following these observations our interest in this condition was keenly aroused and experiments were later undertaken to throw more light upon it. For this purpose a number of groups of ten steers each were placed on test in 1934 at Texas substation No. 7,* Spur, Texas. All were fed a vitamin A-deficient ration consisting of cottonseed meal, cottonseed hulls and white grain with different amounts of alfalfa hay added to the rations for some of the groups, while one group received the vitamin A-deficient ration only.[9] For our purpose here, we are concerned only with group 1, ten steers receiving no alfalfa hay, and with group 2, ten steers receiving 1 lb. of alfalfa hay per head per day in addition to the vitamin A-deficient ration. In this experiment none of the steers in group 2 receiving the alfalfa hay showed signs of ill health during the course of the experiment.

All of the animals were weanling calves taken from their mothers on the range and placed in the feedlot in December. After 120 days on feed the animals on the basal ration in group 1 began to show the first symptoms of a condition that has been recognized as a vitamin A deficiency, the symptoms and lesions of which are indistinguishable from the diseased condition found in commercial feedlots referred to elsewhere. The gain in weight of the animals was arrested and soon a loss in weight occurred. They also showed night blindness, discharge from the eyes and nose, in some cases ulceration of the cornea, rapid respiration during the warmer part of the day and frequent convulsions. Four of them, given 10, 25, 50 and 50 cc., respectively, of cod liver oil made a rapid recovery and again began to gain in weight. The assay of this cod liver oil showed a vitamin A potency of about 550 Sherman-Munsell rat units.

Three other such animals given the same quantity of the same cod liver oil in which the vitamin A had been destroyed continued to grow worse during the following 30 days until they had almost reached a state of exhaustion. At this point one was given 1 lb. of freshly cut green alfalfa, another was given 1 lb. of field-cured alfalfa, and another 0.5 lb. of field-cured alfalfa daily. The field-cured alfalfa had been cut about one week when feeding was started. All three steers recovered on the alfalfa given them, thus showing that 0.5 lb. of the

*In coöperation with the Division of Range Animal Husbandry, Texas Agricultural Experiment Station, College Station, Texas, and the Bureau of Animal Industry, U. S. Department of Agriculture.

[9]Schmidt, H.: Mineral requirements of cattle in the red beds region. Texas Agr. Exp. Sta., Ann. Rpt. (1935), p. 19.

alfalfa used was sufficient to relieve the condition. Another steer was given 10 cc. daily of pure crystalline carotene (having 5,000 international units of vitamin A per gram) dissolved in maize oil, and still another was given 25 cc. of pure crystalline carotene dissolved in maize oil. Both made a rapid recovery, showing that carotene is the substance responsible for the cure and, hence, is the substance lacking in the basal ration. The tenth steer in the lot was given no vitamin A supplement and died on the 179th day of the experiment.

Graph 1 shows the behavior of the weight of the steers in group 2, receiving 1 lb. of alfalfa hay during the whole course of the experiment, and also the weight curves of some of the steers under treatment.

In those steers receiving no carotene or vitamin A, the loss in weight, the disturbances in appetite and the suffering of the animals during hot days became so alarming during the experiment that fear of death was constantly entertained. The results of this experiment, especially the prompt recovery following the administration of pure carotene, could leave no doubt that lack of vitamin A was the sole cause of this condition.

In the next experiments it was planned to supply the vitamin A in the form of carotene, the precursor of vitamin A. This was supplied in the form of alfalfa-leaf meal and fed on the basis of micrograms per 100 lb. of live weight. In the course of the experiment the carotene in the alfalfa-leaf meal was determined every two weeks and the amount adjusted to the body weight of the animal. The first animals were put on a rather low level in order to find just how much was necessary for the maintenance of life and to determine what relationship existed between the amount of carotene required for economical feedlot gains and that of maintenance of health of the animal. It soon became clear that much less carotene was required for economical feedlot gains than for maintenance of health.

Since observations had shown that night blindness is the first noticeable symptom to develop in the syndrome of vitamin A deficiency, this symptom was used as a guide in determining when the animals were de-

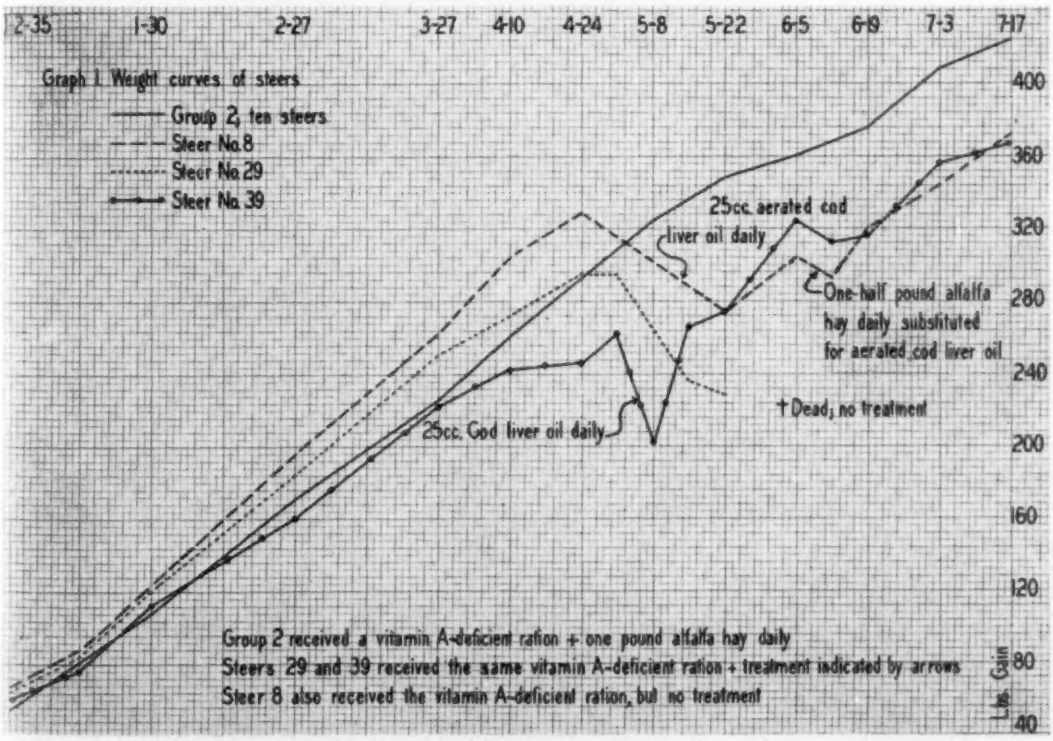

TABLE I—Performance of Steers on 450 γ of Carotene Per 100 lb. of Live Weight. Average Weight at Beginning, 469 lb. Started in Experiment 11-23-36

ANIMAL NO.	DAYS FOR DEPLETION	DAYS ON CAROTENE	GAIN (LB.)	DIED
80	115	485	513	7- 9-38
84	101	145	421	7-27-37
96	108	472	498	6-26-38*
97	108	154	326	8-12-37
99	108	993	746	11-29-39*

*Sacrificed *in extremis*.

pleted of their vitamin A body reserve and symptoms of deficiency could be expected to appear. The steers were placed on a vitamin A-deficient ration and, when depleted, definite amounts of carotene in the form of alfalfa-leaf meal were added to their rations. If we define as a physiologic minimum of carotene requirement of cattle that amount that will prevent night blindness, then in our experiments 2,500 to 3,000 micrograms (γ) of carotene per 100 lb. of body weight daily was required. It does not follow, however, that steers can not be fattened on a lower carotene level, but such level must lie above 450 γ per 100 lb. of live weight, as shown by the following observation.

Five weanling steer calves averaging 469 lb. in weight were placed on a depletion ration in November. After depletion of their body reserves of vitamin A, as indicated by the appearance of night blindness, the time varying from 101 to 115 days, they received 450 γ of carotene daily per 100 lb. of weight and survived on this ration for 145 to 993 days. During this time the individual steers gained from 326 to 746 lb. in body weight and all were quite fat. This shows that if the animal eats well, the ability to store fat is not impaired by a vitamin A deficiency. Their detailed performances are shown in table I.

The clinical behavior of these steers was not different from that observed in dairy cows. After being depleted and night blindness developed, this sympton was not relieved on the carotene level fed, but persisted to the end, and in steers 80 and 99 it progressed to day blindness in one eye. In general, inflammation of the eyes would occur at intervals and corneal opacity varying from small, gray specks to complete involvement of the cornea could be observed. Swelling of the legs, sometimes quite marked and reaching from the hoof to the thigh and shoulder, would occur and remain for weeks and then slowly subside only to return again later.

While the steers were kept on full feed all the time, the appetite varied considerably, ranging all the way from a daily feed consumption of 10 lb. at low ebb to 30 lb. when they were doing well. Toward the end of the survival period the appetite gradually failed and dwindled to a few pounds a day in the course of weeks or longer. During the time of the declining appetite a corresponding loss in weight occurred. A more or less profuse nasal discharge was observed in all steers. This discharge varied from time to time, coming and going much like the swellings of the legs. During the warmer season of the year, and especially during the warmer part of the day, they suffered greatly from the heat. This was manifested by the dribbling of saliva, which was sometimes so profuse that a large body of foam collected on the ground in front of the animal. The steers would frequently pant, often with open mouth and protruded tongue. At night and in the early forenoon these manifestations were not present. The animals would avoid the sun and when forced out of their stall into the hot sun, they would become frantic and make every effort to reach shade. Frequent convulsions were observed in all of the steers. These convulsions would last only a short time, usually less than a minute.

The outstanding symptoms of vitamin A deficiency shown by steers are greatly reduced in severity or entirely absent when the carotene content of the ration is raised to 1000 γ. At this level swellings and nasal discharge are not observed, but a carotene level of 1,250 γ did not completely prevent convulsions. Night blindness is the first symptom that becomes manifest in a vitamin A deficiency and is also the last to disappear in the ascending level of carotene furnished. It is not infrequently present

on a carotene level of 2,500 γ and occasionally also when a level of 3,000 γ is supplied.

ADDITIONAL OBSERVATIONS ON DAIRY CATTLE

Additional experiments to study the vitamin A requirements in dairy cows were undertaken in coöperation with the Division of Dairy Husbandry, beginning in March 1937. Three groups of purebred Jersey heifers about 5 months of age were placed on a vitamin A-deficient ration made up of cottonseed meal, cottonseed hulls and wheat bran. Calcium carbonate was added to supply additional calcium. To this ration enough dehydrated alfalfa-leaf meal was added to supply 1,000, 2,000 and 2,500 γ of carotene and a group of six animals was placed on each of these levels. Here we were interested primarily in the clinical side of the problem. Unfortunately, systematic testing for night blindness was not practiced until the latter part of September 1938, when all of the heifers on the 1,000-γ level were completely night blind. At this time the last animal in the group on the 1,000-γ level had been in experiment 327 days, showing that on this level depletion as measured by night blindness was shorter than this period. At this testing none of the animals on the 2,500-γ level showed night blindness, but at the next test, made two weeks later, and thereafter all of the animals showed night blindness. According to these observations, the six animals in the group on 2,500 γ of carotene became night blind after 340 to 508 days.

None of the cows on the 2,000- and 2,500-γ level showed clinical symptoms of vitamin A deficiency other than night blindness, not even after passing through two and three gestation and lactation periods. On the other hand, the cows on the 1,000-γ level all developed a nasal discharge, swelling of the legs and more or less unthriftiness before pregnancy, but especially following gestation and while in lactation. All in this group were night blind and one developed day blindness in both eyes.

Three of the cows died, two on the 1,000-γ level and one on the 2,000-γ level. Cow 541, on the 2,000-γ level, developed

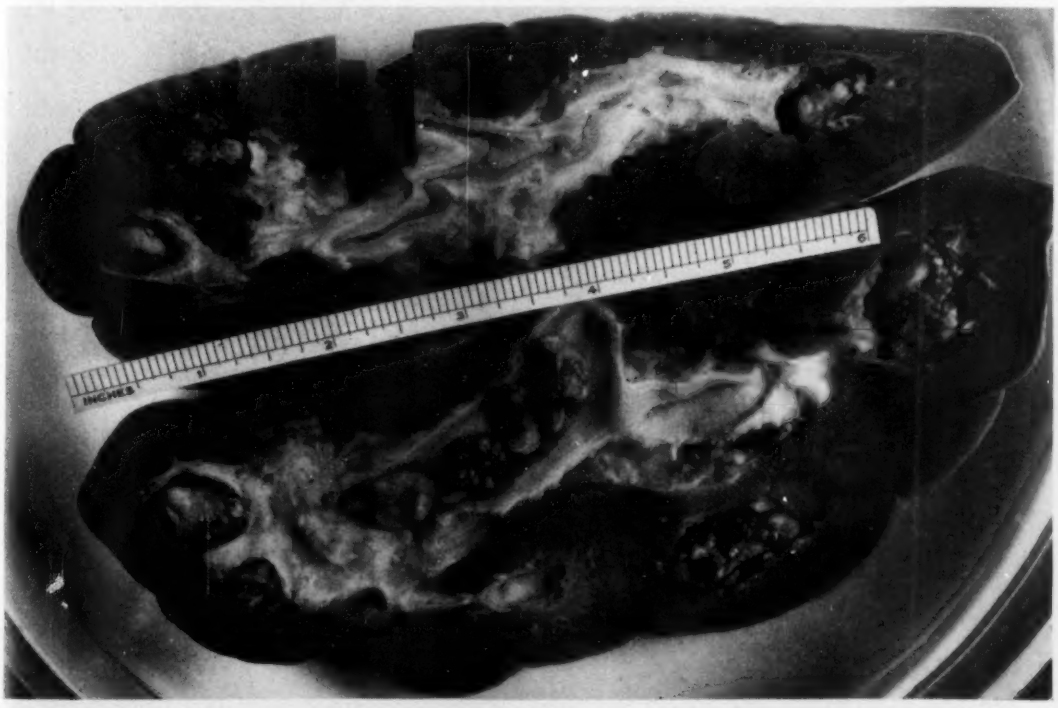

Fig. 3. Kidney of cow 558, showing numerous calculi.

TABLE II—Breeding Performance of Jersey Cows on Various Carotene Levels

Cow No.	γ Carotene per 100 Lb. Body Weight	No. Services for Conception	Calved	Stage of Pregnancy at Parturition (Days)	Remarks
552	1,000	5	10–12–39	253	Born dead
553	1,000	2	4–16–39	229	Born dead
554	1,000	3	1– 4–40	254	Born dead
557*	1,000	11	—	—	—
558*	1,000	4	—	—	—
560	1,000	3	10–15–40	129	Aborted—not brucellosis
539	2,000	3	2–20–39	265	Living, weak; destroyed 24 hrs.
541	2,000	2	1–26–39	268	Born dead
	2,000	1	2–10–40	249	Born dead
542	2,000	1	2–14–39	272	Born dead
	2,000	2	12–29–40	277	Lived 24 hrs.; weak
543	2,000	3	3–27–39	272	Lived 48 hrs.; weak
		1	3–12–40	264	Born dead
		1	5–25–41	277	Lived 5 days
544	2,000	2	3–16–39	264	Born dead
		4	10–15–40	266	Lived; fed cod liver oil
545	2,000	2	2–28–39	273	Born dead
		1	3–11–40	277	Lived 21 days; scours
		1	7–23–41	276	Lived; fed cod liver oil
546	2,500	2	2–16–39	270	Lived 48 hours
		4	1– 3–41	272	Lived 72 hours
547	2,500	2	2–16–39	266	Born dead
		1	2–27–40	270	Lived; fed cod liver oil
548	2,500	2	1–29–39	262	Born dead
549	2,500	1	4–19–39	277	Lived 72 hours
		1	7–11–40	264	Born dead
550	2,500	1	5– 3–39	268	Born dead
		1	8–10–40	277	Lived; fed cod liver oil
559	2,500	1	9–20–39	264	Born dead

*Did not conceive.

metritis following parturition at 249 days of gestation. Other than night blindness she showed no clinical symptoms of vitamin A deficiency. Liver analysis showed 1.9 parts per million spectro vitamin A, indicating that no storage takes place at this level. The cow had been on experiment for 1,060 days.

Cow 558, on 1,000 γ of carotene and in experiment for 1,207 days, had not conceived from four services during this time, was night blind, had frequently shown swelling of the legs and nasal discharge, and became unthrifty towards the end. At autopsy she showed numerous calculi in the kidneys and ureters (fig. 3). No analysis was made of the liver for spectro vitamin A.

Cow 553, on 1,000 γ of carotene and in experiment for 959 days, became unthrifty, had one calf ten months previously, developed marked swelling of the hind legs, became day blind and finally died. The liver contained 1.8 parts per million spectro vitamin A.

Besides the clinical symptoms observed, other manifestations were noted in these animals that merit attention. The growth curve of the animals on the two higher levels was normal, while that of the group on the 1,000-γ level averaged 100 lb. below normal. Reproduction in all groups was poor in quality and quantity, as shown in table II. The six cows on the 1,000-γ level have produced only four calves, all born dead or aborted. The six cows on the 2,000-γ level produced 13 calves, of which six were born dead. Of the seven calves born alive in this group only two could be saved. The six cows on the 2,500-γ level produced ten calves, of which five were born dead, and of the five born alive two could be saved. Of the 27 calves born to these cows, one showed a corneal opacity at birth.

An interesting observation was an abnormality that appeared in one calf each of

two cows, one on 1,000 γ and one on 2,000 γ of carotene. In the calf of the cow on 1,000 γ, a patch of hair grew on the central portion of the cornea of the left eye (fig. 4). This patch of hair was not connected with any part of the skin. In the case of the other cow a similar condition appeared in both eyes. Here the malformation was almost identical in both eyes in so far as patches of skin covered most of the corneae, which is continuous with and like that of the face, and connected with it by a bridge of skin 1 to 1.5 cm. wide at the inner canthi (fig. 5). Other abnormalities also were observed in this calf. Thus, the front end of the maxillae and the intermaxillary bone were bent to the left at an angle of about 30° and the mandible was slightly bent to the same side. The tongue was smaller and shorter than normal; the right forefoot had only one toe and no dewclaws; on the left forefoot one toe was only half the size of its mate. The tibial portion of both hind legs was unduly short; the tibia was only 6 cm. long while the femur measured 14.7 cm. and was abnormal in its lower extremity.

Milk production was likewise poor in all the group. The lactation period was only about one half as long as in normal cows and the milk flow declined rapidly beginning with the fourth to fifth week after freshening instead of continuing at maximum production to the fourth or fifth month.

OBSERVATIONS ON SHEEP AND GOATS

Further experiments for the determination of vitamin A requirements in ruminants were carried on with sheep and Angora goats. For this purpose two classes of animals, namely, yearlings and aged animals, were selected to determine the depletion period and the requirements. It was found that in sheep and goats the picture of vitamin A deficiency varied somewhat from that in cattle. Thus, frequently a loss of appetite, sometimes lasting for several weeks, a loss in weight with an unthrifty wretched appearance and night blindness, were the only symptoms that could be observed. In others a profuse nasal discharge in the form of thick, tenacious strands, or in still others an anemia would develop in addition to the cardinal symptoms already mentioned. Opacity of the cornea was not a constant lesion. Swellings were never observed. No convulsions were observed in the goats. Although these occurred in sheep, the author is still in doubt as to whether they were due to a vitamin A deficiency or a possible vitamin D deficiency. All animals were kept in the barn without access to sunlight and in the early part of the experiments received no additional vitamin D. As a result, rickets developed in a few of the young sheep before vitamin D was added and osteodystrophia developed in some of the young goats. In cattle such convulsions are quite frequent even when they are kept out in the sunlight. The addition of vitamin D to control such convulsions in cattle has not been attempted by the author.

Fig. 4. Left eye of calf from cow 541. Note patch of hair growing on cornea.

The depletion ration selected consisted of 60 per cent cottonseed hulls and 40 per cent concentrates. The concentrate was made up of 75 per cent white kafir meal and 25 per cent cottonseed meal, with 2 per cent calcium carbonate added to this. To this ration the carotene in the form of dehydrated alfalfa-leaf meal was added.

In the first experiments the total survival periods of sheep and goats on a vitamin A-deficient ration were determined. The

TABLE III—Survival Period of Sheep and Goats 1 Year of Age on Vitamin A-Deficient Diet

ANIMAL NO.	DATE ON RATION	SURVIVAL (DAYS)	IMMEDIATE CAUSE OF DEATH	EYE LESIONS	PPM SPECTRO VITAMIN A PER GM. OF LIVER
537S	2-10-36	179	Vitamin A deficiency	—	—
563S	1- 7-37	230	Vitamin A deficiency	+	3.2
564S	1- 7-37	160	Vitamin A deficiency	—	3.2
569S	1- 7-37	183	Vitamin A deficiency	—	3.4
587S	1- 7-37	201	Vitamin A deficiency	—	6.5
588S	1- 7-37	176	Vitamin A deficiency	—	—
589S	1- 7-37	270	Vitamin A deficiency	+	—
615S	2-10-38	214	Vitamin A deficiency	—	2.3
616S	2-10-38	293	Vitamin A deficiency	—	6.3
618S	2-10-38	312	Vitamin A deficiency	—	14.9
570G	1- 7-37	326	Vitamin A deficiency	—	3.5
572G	1- 7-37	255	Vitamin A deficiency	—	3.6
573G	1- 7-37	337	Vitamin A deficiency	+	4.0
574G	1- 7-37	226	Vitamin A deficiency	—	5.1
575G	1- 7-37	357	Vitamin A deficiency	+	—
576G	1- 7-37	350	Vitamin A deficiency	+	—
578G	1- 7-37	420	Vitamin A deficiency	+	3.7
580G	1- 7-37	312	Vitamin A deficiency	—	—
582G	1- 7-37	393	Vitamin A deficiency	+	4.7
583G	1- 7-37	366	Vitamin A deficiency	+	—
581G	1- 7-37	414+	—	+	—
601G	2-10-38	257	Purulent peritonitis	—	—
602G	2-10-38	270	Vitamin A deficiency	—	5.4
603G	2-10-38	283	Vitamin A deficiency	—	—
604G	2-10-38	355	Vitamin A deficiency	—	3.0
605G	2-10-38	265	Vitamin A deficiency	—	19.9
606G	2-10-38	285	Vitamin A deficiency	—	6.1
607G	2-10-38	308+	—	—	—
608G	2-10-38	292+	—	—	—
609G	2-10-38	420	Uremia	—	2.2
610G	2-10-38	355	Vitamin A deficiency	—	4.2
606G	11-25-39	455	Vitamin A deficiency	—	5.7
718G	11-25-39	338	Vitamin A deficiency	+	5.3
719G	11-25-39	384	Vitamin A deficiency	—	3.7
720G	11-25-39	311	Uremia	—	15.7
722G	11-25-39	304	Uremia	—	—
723G	11-25-39	348	Vitamin A deficiency	—	7.0
725G	11-25-39	259	Vitamin A deficiency	—	—

+ = Animal placed on carotene at end of period indicated.
S = Sheep.
G = Goat.

TABLE IV—Survival Period of Aged Sheep and Goats on Vitamin A-Deficient Diet

ANIMAL NO.	DATE ON RATION	SURVIVAL (DAYS)	IMMEDIATE CAUSE OF DEATH	EYE LESIONS	PPM SPECTRO VITAMIN A PER GM. OF LIVER
487S	4- 7-36	779	Vitamin A deficiency	+	18.0
488S	4- 7-36	273	Died unexpectedly		15.2
557S	4- 7-36	290	Vitamin A deficiency	+	85.3
558S	4- 7-36	598	Vitamin A deficiency	+	—
559S	4- 7-36	538	Vitamin A deficiency	+	6.3
507G	7-16-36	351	Vitamin A deficiency	+	9.1
513G	7-16-36	448	Vitamin A deficiency	+	—
551G	7-16-36	422	Vitamin A deficiency	+	—
554G	7-16-36	405	Vitamin A deficiency	—	7.0
469G	7-16-36	787+	—	+	—

S = Sheep.
G = Goat.

number of days that the animal survived on this ration can be seen in table III. It will be noted that in those animals put on as yearlings or short yearlings the depletion time varied somewhat accordingly to the exact seasonal time that they were put on experiment. Thus, it will be noted that the depletion time for goats born around the first of March and placed on experiment the latter part of November was longer than for animals born about the same time and placed on experiment the middle of February and also longer than for those put on experiment the first week in January. This would indicate that young animals grazing during their first summer acquire a definite body store of vitamin A and that little if anything is added to this body reserve after the first frost, which in this latitude occurs around the latter part of November. For every 30 days that the kids were carried on an ordinary winter ration beyond the first frost, the survival period, upon placing the animals on a vitamin A-deficient ration, was about 30 days less than for animals placed on the depletion ration at the time of the first frost. The survival period of sheep of a similar age or about one month older at the corresponding calendar time was considerably less than for goats in the same experiment. In goats this survival period varied from 226 to 455 days, with the majority falling between 255 and 420 days, whereas in sheep the survival period varied from 160 to 312 days, with the majority falling between 176 and 293 days. (See table III.) Our data on the survival period of aged sheep and goats are rather scarce, but those available indicate that this period is about the same for aged sheep as for aged goats, varying from around 350 to 790 days (table IV).

When night blindness is used as the yardstick, the determination of the depletion period in sheep and goats is much more difficult than it is in cattle. Sheep and goats, in the author's experience, are more difficult to test for night blindness than cattle and often one is left in doubt as to the degree of night blindness present unless the affection has progressed almost to the extreme. The author has found no other clinical guide that is satisfactory to accurately determine the depletion period. Un-

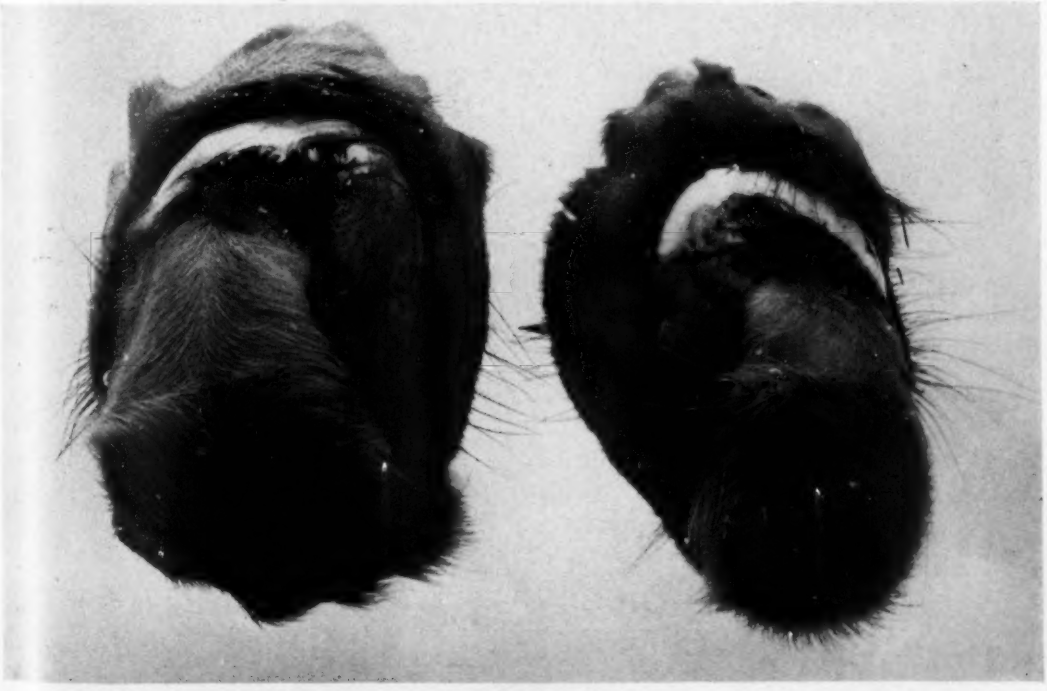

Fig. 5. Eyes of calf from cow 549. Note skin attached to cornea.

TABLE V—Vitamin A Requirements for Sheep

ANIMAL NO.	APPROXIMATE DAYS TO DEPLETION	γ CAROTENE PER CWT. BODY WEIGHT AFTER DEPLETION	DAYS ON CAROTENE	REMARKS
534	180	650	1360	Exp. terminated; sheep fat, healthy
536	180	650	1017	Death unexpected 2 wks. before due to lamb
562	150	650	951	Exp. terminated; sheep fat, healthy
586	150	650	951	Exp. terminated; sheep fat, healthy
560*	151	650	691	Died vitamin A deficiency?
486†	258	650	25	Died vitamin A deficiency?
611	256	750	496	Exp. terminated; sheep fat, healthy
612	403	750	412	Exp. terminated; sheep fat, healthy
613	389	750	451	Died—uremia
614	256	750	524	Exp. terminated; sheep fat, healthy
617	355	750	408	Died—uremia
619	355	750	454	Exp. terminated; sheep fat, healthy
620	285	750	211	Died vitamin A deficiency (?)
641	72	750	441	Exp. terminated; sheep fat, healthy
642	72	750	399	Exp. terminated; sheep fat, healthy
643	72	750	399	Exp. terminated; sheep fat, healthy
644	72	750	399	Exp. terminated; sheep fat, healthy
645	92	750	399	Exp. terminated; sheep fat, healthy
483†	286	750	405	Exp. terminated; sheep fat, healthy

*2 years old; all others 1 year old when placed on experiment.
†Aged sheep.

der these difficulties the depletion period was determined as near as possible and thereafter a number of animals placed on carotene to study their requirements for this substance.

Table V gives the results obtained in condensed form. In explanation, it might be said that all animals on the levels indicated were considered to be night blind, as near as could be determined during the whole period of the experiment, so that 650 and 750 γ of carotene per 100 lb. of body weight did not suppress night blindness. The table shows, however, that this did not keep the sheep from developing into excellent physical specimens. The time that these animals were kept on the indicated levels was considered sufficiently long; hence, any breakdown in the health of the animals from a lack of vitamin A was considered remote. During this experiment three animals died from a questionable vitamin A deficiency. Two others died from uremia following occlusion of the urethra by urinary calculi. Whether the deaths of the first three were really due to a vitamin A deficiency remains an open question, because microscopic examination of the material has not yet been completed. One animal died unexpectedly after having been on a level of 650 γ of carotene per 100 lb. of body weight for 1,017 days. It would appear from these findings that a level of 750 γ of carotene per 100 lb. of live weight may be sufficient under ordinary circumstances, but in case of stress may be too low.

A number of the animals listed in table V were changed to a higher level, varying from 2,500 to 4,000 γ after the results shown had been obtained and were continued thereon for a year in order to note the influence of a higher carotene level upon night blindness. No such influence could be detected. This would mean that either our method of detecting night blindness is too crude to give dependable results or that night blindness, once it has existed for the periods indicated in table V, is not influenced by the higher level of carotene used.

THE RELATION OF VITAMIN A DEFICIENCY TO UROLITHIASIS IN GOATS

During the progress of these experiments our attention was repeatedly drawn to the fact that on autopsy some of our animals would show encrustation of the kidneys with mineral deposits (fig. 6), or kidney

Fig. 6. Kidneys of sheep 487, showing incrustation with mineral deposits.

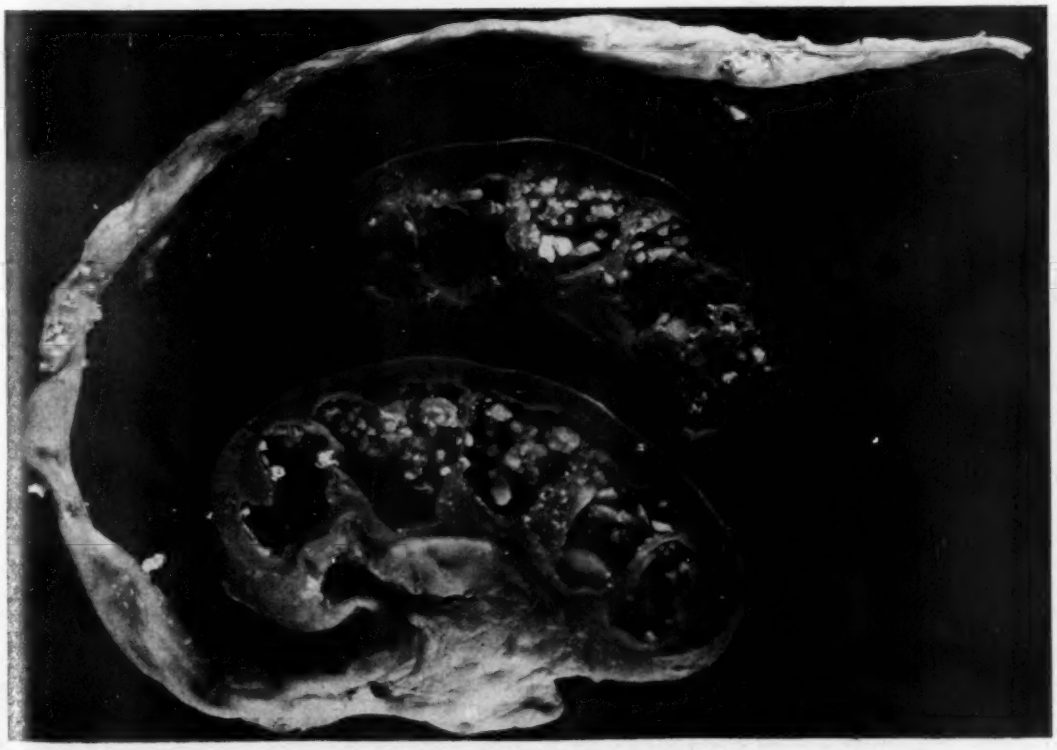

Fig. 7. Kidneys of sheep 642, showing kidney stones.

TABLE VI—Vitamin A Deficiency and Urolithiasis in Castrated Male Yearling Goats on 2,500 γ Carotene and Controls Without Carotene

Animal No.	Urethral Occlusion	Days to First Urethral Occlusion	Days Survived on Experiment	Uroliths	Remarks
598G	—	—	411	—	Destroyed; osteodystrophia fibrosa
710	+	381	627*		
711	—	—	426	—	Destroyed; osteodystrophia fibrosa
712	+	188	200	+	
713	—	—	598	—	Destroyed; osteodystrophia fibrosa
716			627*		
724			627*		
			Controls—No Carotene		
606G	—	—	456	—	
718	+	140	339	+	Many large stones in kidney and ureter, none in bladder
719	+	307	385	—	
720	+	133	313	+	
722	+	247	305	+	
723	+	343	352	+	
725	—	—	260	—	Polyurea; recumbent for 3 weeks preceding death

*Animal is still in experiment.

stones (fig. 7) or calculi in the urinary bladder. Even before death some would show urethral occlusion, which was frequently removed spontaneously. These observations prompted us to study the relation between vitamin A and uroliths more carefully.

In order to throw more light upon this phase of the investigation two experiments were run to determine what effect a vitamin A-deficient ration would have upon the formation of urinary calculi. The ration used was the same as in all preceding experiments, with the exception that when osteodystrophia fibrosa developed in some of the animals, 0.2 Gm. of irradiated yeast containing 3,200,000 USP units of vitamin D per pound was given every other day. In each instance one half of the animals were placed on a definite carotene level while the other half served as controls, being fed identical rations otherwise. In each case the animals were as nearly alike as possible, having run in the same pasture during all of their one year of life.

In the first experiment, begun January 1, 1940, annotated in table VI, seven animals were placed on 2,500 γ of carotene per 100 lb. of body weight. Of these seven animals two developed urethral occlusion, one after 188 days and the other after 381 days. The first of these two, goat 712, died at the end of 200 days on experiment; the other is still living after 627 days on the experiment. Three of the animals developed osteodystrophia fibrosa and were destroyed after 411, 426 and 598 days in experiment; the remaining three are still being continued on the experiment. Of the four animals that have thus been eliminated from the experiment, only goat 712 showed uroliths. Of the seven control animals five developed urethral occlusion and four of the seven showed uroliths *post mortem*, all seven having died.

In the second experiment, begun January 1, 1941 (table VII), 13 animals were placed on the same ration as those in the first experiment, but this time six of them were given 3,500 γ of carotene per 100 lb. of body weight in their ration. The seven control animals in this experiment all showed urethral occlusion at one time or another and six of them have died. Only one showed definite uroliths at autopsy and in one the urethra was blocked by a soft

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cheesy material, which was also contained in the urinary bladder in a large amount. It is interesting to note that five of the six animals which died out of this group so far, died from a ruptured urinary bladder.

Of the seven animals on 3,500 γ of carotene, two died with ruptured bladder, but only one of them showed uroliths at the time of death. The other five animals are still surviving after a period of 225 days on experiment. It is of interest to note that all of them died too early for their body reserve of vitamin A to have been depleted, as proved by previous experiments. Our data are not complete as to the origin of the material occluding the urethra, but it is believed that this material is composed of cast-off cornified epithelial cells which are desquamated in large masses as a result of vitamin A deficiency (fig. 8). In this connection, it is of interest to note the number of days required for the first urinary occlusion to develop. In many instances urinary occlusions would repeatedly be relieved only to develop again later. The days indicated in the table are those elapsed up to the time the first urinary occlusion was observed in the different animals, subsequent occlusions not being entered in the table. The time in which urinary occlusion developed ranged from 140 to 343 days in the first experiment and from 112 to 176 days in the second experiment for the control animals, whereas in the animals receiving carotene none developed in the first experiment, although in the second experiment two developed the condition after 188 and 381 days, respectively.

SOURCE OF VITAMIN A FOR RUMINANTS

The sole source of vitamin A for ruminants under natural conditions is the carotene in their plant food. Green plants contain an abundance of carotene, but as soon as the plant dies, be it when it is cut for hay or from other causes, the destruction of the carotene begins and proceeds at a rapid rate, especially in the presence of light. Thus, it has been shown that in alfalfa, cut for hay and exposed to the sun to dry, around 98 per cent of the carotene is destroyed during the first 48 hours of exposure. Rapid drying at high temperatures of freshly cut green alfalfa preserves much of the carotene and such an artificially dried alfalfa hay when fresh contains several hundred micrograms of carotene per gram. A 1,000-lb. steer at a carotene level of 3,000

TABLE VII—Vitamin A Deficiency and Urolithiasis in Castrated Male Yearling Goats on 3,500 γ Carotene and Controls Without Carotene

ANIMAL NO.	URETHRAL OCCLUSION	DAYS TO FIRST URETHRAL OCCLUSION	DAYS SURVIVED ON EXPERMENT	UROLITHS	REMARKS
609G	+	165	171	+	Ruptured bladder
616G			225*		
751			225*		
752			225*		
753			225*		
754	+	119	130	—	Ruptured bladder
			CONTROLS—NO CAROTENE		
635G	+	142	157	+ (?)	Cheesy material in bladder and urethra; bladder intact
637G	+	164	172	—	Ruptured bladder
755	+	169	175	—	Ruptured bladder
757	+	176	184	+	Ruptured bladder
760	+	112	225*	—	
761	+	115	168	—	Ruptured bladder
762	+	174	165	—	Ruptured bladder

*Animal is still in experiment.

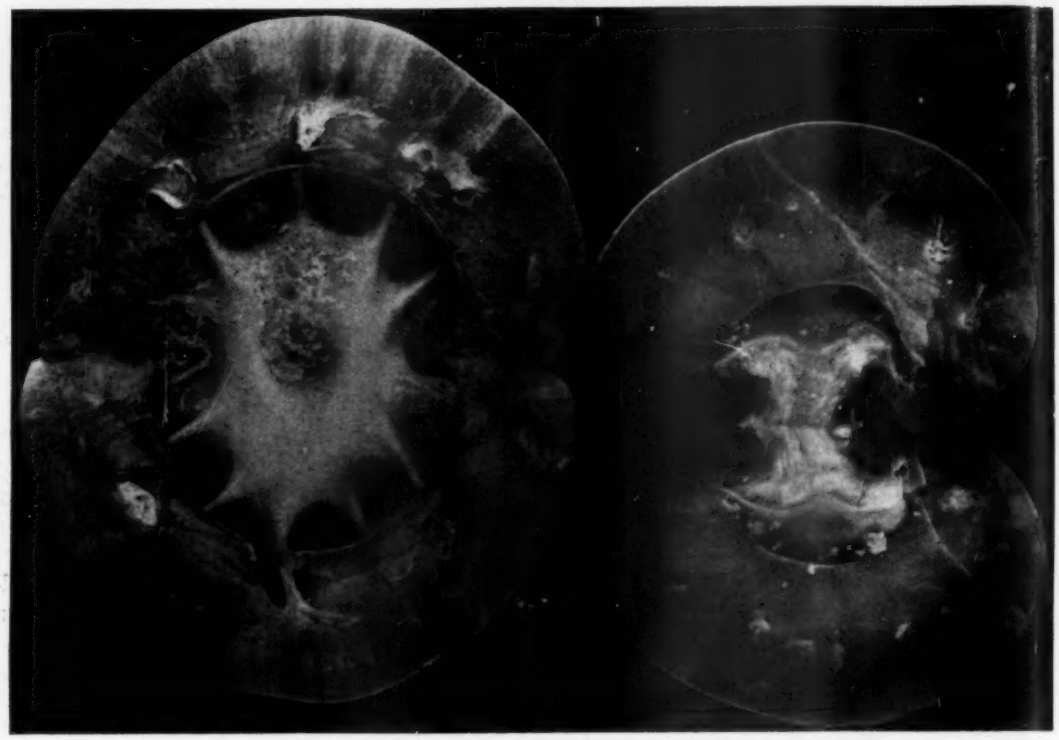

Fig. 8. Kidneys of goat 719, showing desquamated material in pelvis.

γ per 100 lb. of body weight would require only 150 Gm. per day of such hay for the maintenance of perfect health.

Plants curing on the stem, whether from drouth or the approach of maturity, or when cut for hay or fodder or made into silage, lose the greater part or practically all of the carotene so that the carotene content may run so low as to be almost negligible and unable to cover the requirements of an animal for long.

It is also known, as revealed by liver analysis, that the young is born without a vitamin store and must receive carotene or vitamin A soon after birth. It finds an abundant supply of it in the colostrum of its mother and in milk. When the young begins to graze, it finds an abundant supply in the green plants. Part of the carotene it takes up is used to maintain its body functions, while part of the surplus is stored in the liver.

As the animal advances in age, the amount of storage in the liver increases and is reflected in the time required to deplete the animal. That it also depends upon the condition of the range goes without saying. According to the data presented, a young animal going through a protracted drouth in the late summer may well become depleted before winter is over, or even in the feedlot, unless carotene is available.

The author has observed a case where some 300 three-year-old steers were placed in the feedlot in late fall of a drouth year and fed sorghum roughage and milo grain. The steers were finished in due time, but a break in the market caused them to be held over pending improvement in the market. After 240 days in the feedlot about 60 of the steers began to develop swelling of the legs, loss of appetite and weight and night blindness, and one of them died. Analysis of the liver of this steer showed only 3.7 parts per million spectro vitamin A. Another case observed was that of a dairy farmer raising his calves in a dry lot on skimmed milk and sorghum roughage with a little grain. Three age groups were involved in the lot, the oldest 4 months and the young-

est about 2 months. All showed night blindness, a number of them corneal opacity and two ulceration of the cornea. The addition of alfalfa to the ration relieved the condition.

SUMMARY

Observations on vitamin A deficiency in cattle, sheep and goats in both feedlot and experimental animals are herein reported. The syndrome developing in commercial feedlots in fattening cattle fed exclusively on cottonseed hulls and cottonseed meal was shown to be a pure vitamin A deficiency readily curable by the feeding of sufficient crystalline carotene in maize oil. Cod liver oil, green feed or feed containing an appreciable amount of carotene is equally effective in relieving the condition.

As observed, the deficiency manifests itself in cattle in night blindness, corneal opacity and ulceration, convulsions, swelling of the legs and other parts of the body, nasal discharge, unpliable skin, failure of reproduction, panting and drooling in hot weather, sensitiveness to heat and loss in weight and appetite. If the deficiency is severe enough, it terminates in death.

The time required for the deficiency to develop depends upon the body reserves, which in turn depend upon the age of the animal and the amount of carotene contained in its food. Young animals are, therefore, more rapidly depleted than old ones. The amount of carotene required by cattle for continued maintenance of life lies above 450γ per 100 lb. of live weight. Five Hereford steers placed on this amount of carotene after depletion became fat during the following 145 to 993 days on this level and yet all five eventually succumbed to a vitamin A deficiency. This shows that a greater amount of carotene is required for maintenance of health than for fattening.

For Jersey cattle the amount of carotene required for maintenance of health lies above 1,000γ per 100 lb. of body weight. A carotene level of 2,000γ did not prevent night blindness in maturing Jersey heifers, and when a carotene level of 1,000γ was fed to the same class of animals, night blindness, marked nasal discharge and swellings of the legs developed. Day blindness developed in one out of four cows on the 1,000-γ level following gestation and lactation. This animal became progressively unthrifty and finally died from a vitamin A deficiency. Reproduction on carotene levels of 1,000, 2,000 and 2,500γ was not successful and lactation was much below normal.

The survival period of mature sheep on a carotene-free ration varied from 273 to 779 days, and of mature goats from 351 to more than 787 days. Of six sheep placed on 650γ of carotene per 100 lb. of live weight, four survived 951 days or longer when the experiment was discontinued, although one of these died unexpectedly on the 1,017th day. Of 13 sheep placed on 750γ of carotene, 10 survived until the experiment was terminated 399 to 524 days later; two died from uremia and one probably from a vitamin A deficiency.

Of 14 castrated yearling male goats on a vitamin A-free diet, five developed uroliths and twelve suffered from urethral occlusion one or more times, with five dying from a ruptured urinary bladder. This was also the cause of death in two animals getting 3,500γ of carotene, but only one of these two showed uroliths. Uroliths also were observed in one out of seven animals on 2,500γ of carotene. In those cases of urethral occlusion where no uroliths were found, the occlusion was probably caused by detritus from the pelvis of the kidney or other parts of the urinary tract.

The Relation of Brucella Abortus Infection to the Vitamin A Content of Fetal Livers*

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THE FINDING OF the causative organism of infectious abortion in clumps in the cells of the fetal membranes was reported by Bang,[1] by the British Board of Agriculture and Fisheries departmental committee on epizoötic abortion[2] and by Zwick and Zeller.[3]

REVIEW OF PREVIOUS INVESTIGATIONS

Theobald Smith[4] described with illustrations the characteristic localization of *Brucella abortus* in the bovine fetal membranes. The epithelial covering of the chorion is a characteristic habitat of the organism. This layer of cells faces and is in intimate contact with the uterine mucosa in the intercotyledonous areas. It continues over the villi of the fetal cotyledons, which dip into the depressions, or sulci, of the maternal cotyledons.

These cells, which were demonstrated individually or in series to become densely filled with the organism, become altered in various ways as a result of the invasion. The cells of the chorion and margin of the cotyledons and outermost villi were found to be invaded. The bulk of the villi were found to be free from the nests of bacteria but still showed marked alterations. The author stated, "It may be maintained that the cell parasitism is not an active disease process but rather secondary to it and operating only in cells partly or wholly devitalized." The epithelial cells of the uterine mucosa as well as those of the amnion were not found to have been invaded. The pathogenic significance of the condition could not be properly evaluated, but the author assumed that it could be one of a series of localizations in the membranes by which the organism gains an advantage over the host. Another localization was found to be in the walls of the blood vessels of the chorion. Such multiplication of bacteria in cells not having phagocytic functions was mentioned as having been demonstrated in leprosy, syphilis and other diseases.

More recently studies by Mason[5] in the rat showed the alterations produced in the gravid uterus by vitamin A and E deficiencies. In this work control rats and others deficient in A, deficient in both A and E, and deficient in E were allowed to become pregnant. Macroscopic and microscopic studies of the effects of the deficiencies in the developing young and the placental membranes were made. In the animals deficient in vitamin A with or without E deficiency, on the ninth to the fifteenth day of pregnancy there appeared, macroscopically, implantation sites containing dead and resorbing fetuses intermingled with others that were normal grossly.

Histologically there were even greater differences in the resorbing fetal sites. Gross changes were not marked before the eleventh day, but microscopic sections showed extensive changes on the eighth to the tenth day. Most characteristic of these changes were areas of focal necrosis in the maternal decidua adjacent to the fetal trophoblast. The character of these changes was essentially the same in all instances, but they varied in extent and time of appearance. The cellular necrosis appeared to be the result of an infective process, with gram-positive organisms demonstrable. In some instances the area of focal infection and necrosis involved the decidual tissue to a point of rupture into the uterine cavity, which is well shown in photomicrographs accompanying the data. Also, there developed rupture of the vascular sinuses of the maternal decidua and the fetal trophoblast with extensive hemorrhage into the uterine cavity. Many fetal sites showing no necrosis were retarded in development that appeared to involve the maternal decidua much more than the fetal tissues, and fetal differentiation was not significantly retarded.

The decidual injury invariably preceded alterations in the fetal tissue when only vitamin A deficiency existed. The fetal injury consisted of general disintegration from lack of nutrition following placental injury. The author could not determine that it was accompanied by in-

*From the Division of Animal Husbandry, College of Agriculture, University of California. The experimental work reported herein became coöperative with the Bureau of Animal Industry, U. S. Department of Agriculture, July 1, 1929.

[1]Bang, B.: Zeit. f. Thier. Med., 1 (1897), p. 241.

[2]Great Britain Board of Agr. & Fisheries, Rpt. Dept. Comm. on Epizoötic Abortion (London, 1909), part 1.

[3]Zwick, W., and Zeller, H.: Arb. k. Gsndhtsamte, xliii (1913), p. 1.

[4]Smith, T.: J. Exp. Med., xxix (1919), pp. 451-456.

[5]Mason, K. E.: Am. J. Anat., lvii (1935), pp. 303-349.

fective processes until breakdown of the fetal membranes and fetal tissues had occurred. In vitamin E-deficient rats there was early disintegration of the fetal tissues and later degeneration of the decidua with no infection and cellular necrosis.

This was found previously by Evans and Burr,[6] who stated, "After the death of the fetus in E-free cases, which takes place at some time during the thirteenth day of gestation, the placenta continues to live and to undergo changes which to some extent might be said to represent efforts to follow the normal plan of development."

In Mason's work, if both vitamin A and E deficiencies existed, fetal disintegration from the lack of E preceded or appeared simultaneously with infection and necrosis of the decidual tissue from lack of vitamin A.

Green, Pindar, Davis and Mellanby[7] also observed sepsis of the generative tract in pregnant rats on vitamin A-deficient diets. Increased intake of vitamin A during the last few weeks of pregnancy in man was demonstrated by them to be effective in reducing the incidence of puerperal infection.

Low vitamin A can effect fetal cell differentiation, as demonstrated by Hale[8] in the pig. He reported having a gilt low in vitamin A during the early stages of gestation. The intake was then increased so that gestation could be completed, and the animal farrowed eleven pigs, none of which had eyeballs.

In work with cattle Hart and Guilbert[9] showed that vitamin A could be the cause of premature termination of pregnancy under dry-range conditions. It was further shown that the differential diagnosis between this condition and infectious abortion could not be recognized clinically. It was stated, "Blood samples must be taken from the aborting cows and the serum tested for the presence of agglutinins with *Brucella abortus* antigen. If any positive reactors are found, infectious abortion is present and must be eliminated before the identity of the vitamin A deficiency can be established. In testing for vitamin A deficiency liver samples from the aborted fetuses or from dead calves are forwarded to the nutrition laboratory to be examined chemically by the colorimetric test. A negative chemical test if the agglutination tests of the cows are negative indicates that vitamin A deficiency is causing the trouble; if the agglutination tests are positive vitamin A deficiency may be present along with abortion infection."

In 1937, Haring and Traum[10] reported results of exposing vaccinated and control cattle to virulent *Br. abortus* organisms. Their group-4 animals consisted of seven vaccinated and seven controls which were kept continuously on pasture. Weather conditions were such during the summer and fall that the natural vegetation became unusually dry and bleached. Rains were late and were followed by such cool weather that no green feed developed until after the first of January 1937. Alfalfa hay was fed sparingly two or three times weekly from November to February. All of the controls and three of the seven vaccinated animals aborted and the organism was isolated from the uterus in all cases. They were all first-calf heifers while the four vaccinated animals that carried their calves to term had previously borne calves and, therefore, were older and had an opportunity for greater vitamin A storage. Their uteri and milk were free from the abortion organism.

The antimony-trichloride test on the liver tissues of some of the aborted fetuses was completely negative. This test made on other calves born to cows in the vicinity and fed alfalfa *ad libitum* showed a relatively high content of vitamin A. The authors stated, "It is probable that a combination of the effects of *Br. abortus* infection and low intake of vitamin A and possibly other dietary essentials were responsible for the abortions in Group 4."

EXPERIMENTAL PROCEDURE

We became interested in the effect of infection with *Br. abortus* on the vitamin A content of the livers of aborted fetuses. During 1938 and 1939, H. E. Kingman, Jr., junior veterinarian in the U. S. Bureau of Animal Industry, was carrying on field experiments in California to test the immunizing value of strain 19 abortion vaccine. This laboratory agreed to check for him the possibility that vitamin A deficiency might be involved in occasional abortions occurring in vaccinated cows. He also furnished us liver tissue from fetuses aborted from positive reacting, nonvaccinated cows. All of the samples from aborted fetuses attributable to *Br. abortus* infection were from dairy cows in the irrigated valley section around Modesto, Calif. These cattle had access to an abundance of green pasture or alfalfa hay. Vitamin A deficiency in the diet of the aborting cows was, therefore, ruled out. A series of liver samples from newborn calves or fetuses from normal animals under like feed conditions is presented for comparison. The liver samples were either refrigerated as soon as obtained, then minced and placed in a 5 per cent solution of alcoholic KOH, or were at once placed in the alcoholic KOH solution. The liver samples weighed approximately 20 Gm. The vitamin A content of livers has been found to be reasonably stable during transit to the laboratory under these conditions. Upon arrival at the laboratory the

[6] Evans, H. M., and Burr, G. O.: Mem. Univ. California, viii (1927), pp. 1-176.
[7] Green, H. N., Pindar, D., Davis, G., and Mellanby, E.: British Med. J., ii (1931), pp. 595-598.
[8] Hale, F.: J. Heredity, xxiv (1933), p. 105.
[9] Hart, G. H., and Guilbert, H. R.: California Agr. Exp. Sta. Bul. 560 (1933).
[10] Haring, C. M., and Traum, J.: J. Agr. Res., lv (1937), pp. 117-128.

TABLE I—Vitamin A Content of Fetal Livers as Affected by Brucella Abortus Infection

DATE OF ABORTION	DATE OF LIVER ANALYSIS	AGGLUTINATION TEST OF COW	APPROX. AGE OF FETUS (MONTHS)	VITAMIN A, BLUE UNITS PER GRAM	REMARKS
2/24/38	3/ 2/38	++++	7	2.5	Cow negative Oct. 1, 1937
2/26/38	3/ 2/38	++++	7	1.0	Sample refrigerated
3/ 1/38	3/ 8/38	++++	?	0.0	Sample frozen, then placed in alcoholic KOH for shipping
3/25/38	3/29/38	++++	7	5.0	Hydrocephalic calf
3/27/38	3/30/38	++++	5½	Trace	Sample placed in alcoholic KOH and refrigerated within few hours of abortion
3/29/38	3/31/38	No test	7	2.0	Born alive; died within a few minutes; sample refrigerated overnight, then placed in alcoholic KOH
4/12/38	4/14/38	++++	6½	1.0	Sample immediately placed in alcoholic KOH
4/17/38	4/20/38	++++	8½	0.0	Calf born alive; died immediately
4/11/38	4/20/38	++++	7½	Trace	Sample in alcoholic KOH at ranch to 4/18; some evidence of decomposition
6/16/38	6/23/38	No test	?	0.0	Same ranch as above; no record of agglutination test
11/13/38	11/18/38	++++	6	Trace	Cow blood contained 6.7 B. U. vitamin A per cc.; high in carotene
11/15/38	11/18/38	++++	8	4.0	Cow born 3/13/36; vaccinated strain 19 10/14/36: 4/6/37 + + — —; 5/4/38 + — — —; 10/21/38 ++++; blood contained 2.2 B. U./cc.
2/ 7/39	2/11/39	++++	8½	0.0	Cow vaccinated; blood gave positive vitamin A test; green feed abundant
				LIVER SAMPLES FROM NORMAL ANIMALS	
(Date Killed)					
3/16/38	3/18/38		8	50.0	Normal fetus from slaughter house
3/28/38	3/30/38		8	100.0	Normal fetus, cow T. B. reactor
10/25/38	10/27/38		4–5	12.0	1st calf heifer, T. B. reactor, slaughtered
10/25/38	10/27/38		5	50.0	1st calf heifer, T. B. reactor, slaughtered
10/25/38	10/27/38		5	17.0	1st calf heifer, T. B. reactor, slaughtered
Data from Guilbert and Hart[11]			7½	100.0	Dam received 75 cc. cod liver oil daily
			8½	12	Died at birth 10 days premature
			Term	14	Killed 18 hours after normal birth
			Term	10	Killed 36 hours after normal birth
			Term	5	Killed 3 days after normal birth
			Term	25	Born dead at term
			Term	42	Born dead at term

samples were digested by heating and the vitamin A determined according to the procedure outlined by Guilbert and Hart.[11] The data are presented in table I.

RESULTS

Seven of the 13 liver samples of the aborted fetuses from positive-reacting dams were either negative for the vitamin A test or contained only a trace and the remaining ones were low. Similar results were obtained on a series of nine aborted fetuses from both vaccinated and nonvaccinated cows which were infected with virulent organisms. These liver samples were submitted to us by Haring and Traum of the Division of Veterinary Science, University of California. In contrast, the samples from normal animals varied from 5 to 100 blue units per gram. It is apparent that Brucella infection impairs the transfer of vitamin A from the maternal blood to the fetus, and the results serve to emphasize again the necessity of ruling out

[11]Guilbert, H. R., and Hart, G. H.: J. Nutri., viii (1934), pp. 25-44.

infectious causes of abortion before diagnosing vitamin A deficiency as the cause of losses. If infection has been eliminated, a negative vitamin A blood test of the mother and a negative test of the fetal liver with a history of the dam's subsisting for several months on dry, bleached forage or other low-carotene rations make a diagnosis of vitamin A deficiency reasonably certain.

The blood of the dam should be collected without allowing access to feed other than the regular diet, as ingested carotene quickly appears in the blood stream.

The possibility of infection with *Vibrio fetus* or trichomonads, causing vitamin A depletion of fetal livers, also must be considered. On this question no data are available at present.

DISCUSSION

In uncomplicated vitamin A deficiency resulting in abortion or in weak, nonviable calves, vitamin A is not demonstrable in the livers of the young. Calves and lambs from mothers receiving deficient diets that appeared perfectly normal at birth, on the other hand, always have given positive tests for vitamin A when the livers were examined. This is evidence that a negative liver test represents a condition that is incompatible with life in the young.

It is well known that the vitamin A and carotene content of the blood serum decreases rapidly when animals are changed from high to low intake and that this change is almost immediately reflected in the milk. In man, mild decrease in dark adaptation has been shown to occur within 30 days after change from prolonged high vitamin A intake to a vitamin A-deficient diet. Presumably, high storage had been established. In this case one might postulate that conservation of reserves restricted the amount liberated in the blood stream to an extent less than required for optimal dark adaptation.

The fetus utilizes vitamin A almost as rapidly as this substance passes the placental filter and, consequently, storage in the newborn is low regardless of the intake of the mother. An abundant supply is naturally provided in the colostrum when pregnant animals receive a normal diet. In a severe natural outbreak of vitamin A deficiency under our purview (Hart and Guilbert[9]) it was the practice on the affected grain ranch to bring dairy calves from an alfalfa section to raise for veal. These calves, having been born with normal liver reserve and having received colostrum, were able to subsist on the deficient nurse cows of the grain ranch for about seven weeks before symptoms appeared. Calves from the cows on the grain ranch were born dead or died within a few days after birth.

In vitamin A deficiency there is evidence, at least in the rat, that placental alteration occurs well in advance of fetal death. The latter is due to nutrients being shut off by the placental changes and the supply of vitamin A would be utilized before death. Calves may be born prematurely while still alive or at term and very weak.

The frequency with which abortions occur in the cow while the fetus is still alive is proof that expulsive contractions are not dependent on a dead fetus acting as a foreign body. The placental injury that occurs both in vitamin A deficiency and Brucella infection may be a more logical explanation of the phenomenon, and hormonal interchange may be involved.

Consideration of the nature and mode of action of vitamin A deficiency and of Brucella infection suggests the following hypotheses regarding possible interplay between the two conditions:

1) Involvement of the fetal membranes by Brucella organisms may restrict the transfer of vitamin A to the fetus and membranes and thereby decrease vitality or cause death.

2) Vitamin A deficiency with its coincidental alteration in placental cells, vulnerable to deficiency because they are developing rapidly, could hasten the invasion and effect of the Brucella infection, which exhibits tropism for these tissues.

3) The resistance to infection produced

The Effect of Parturition on the Blood Picture of Cows in Health and During Infection with Brucella Abortus*

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A STUDY WAS initiated in this laboratory to determine the normal variation in the total number of erythrocytes and leucocytes per unit volume, and in the percentages of the different types of leucocytes, in the blood of individual healthy cattle, in order to be able to measure the effect of infection with *Brucella abortus* on the blood cells. The correlation coefficients calculated from the entire group of observations, as yet unpublished, on the healthy cows revealed no significant relationship between the progression of pregnancy with either the changes in number of the red or white cells per unit volume or the percentages of the different types of leucocytes. However, when the data taken during the parturient period were examined, definite deviations from the normal variation in cell numbers were evident, particularly in the period immediately following calving. This observation led to an analysis of the variation of the cellular counts made preceding and following parturition to determine whether consistent changes had occurred.

*From the Department of Veterinary Science and the Department of Genetics (paper No. 273), University of Wisconsin, in coöperation with the Bureau of Animal Industry, U. S. Department of Agriculture.

(*Continued from preceding page*)

in cattle by *Brucella abortus* strain 19 vaccine is a relative immunity which can be overcome by sufficiently severe natural infection. It is possible that either a degree of vitamin A deficiency or exposure to natural Brucella infection, which could not cause abortion when acting alone, might effect actual expulsion of the fetus in vaccinated animals when acting together.

4) Critical experiments to prove or disprove these possibilities have not been carried out.

MATERIALS AND METHODS

The procedures used in determining the total number of erythrocytes and leucocytes per cubic millimeter of blood and the percentage of the different types of leucocytes have been previously described[1] in detail. Standard methods were used for the enumeration of the total red and white cells. The supravital staining method, as described by Sabin,[2] was employed for the differential classification of the leucocytes.

Twenty-four cows of the Holstein-Friesian breed were used in this study. They varied in age from 18 months to 8 years at the beginning of this experiment, 20 being heifers in their first pregnancy. In most cases the blood counts* were made on each of the cows at weekly intervals, with more frequent observations occasionally on some individuals. The data include observations on 20 of the cows through two normal parturitions, on three cows through one normal period, and on 19 of the same individuals through an infected (artificially induced) pregnancy. Of the last-named group, five carried their calves to term while the remaining 14 aborted fetuses of varying ages.

The observations on this herd were divided into two groups, depending upon the classification of the animals. The "normal"† group was composed of the animals which had not yet been exposed to Bang's disease; the same animals made up the "infected" group following their artificial exposure to *Br. abortus*. All of the results for the "normal" group were arranged in a chart designed to locate each particular count of an individual on the exact day before or after parturition on which the blood sample was taken. For example, cow lc calved May 10, 1936. Counts were made on

*Blood count or blood picture, as used in this paper, refers to the enumeration of the total numbers of erythrocytes and leucocytes per cubic millimeter, and the differential classification of the leucocytes.

†"Normal" refers only to the freedom from infection with *Br. abortus*. Mastitis and, undoubtedly, other commonly occurring infections were present from time to time.

[1]Bell, F. N., and Irwin, M. R.: Studies on the variation of the blood cells of cattle in health and during Brucella infections. J. Inf. Dis., lxiii (1938), p. 251.

[2]Sabin, Florence R.: Studies of living human blood cells. Bul. Johns Hopkins Hosp., xxxiv (1923), p. 277.

her blood on April 7, April 14, April 21, April 28, May 5, May 12, May 19, May 26, June 2 and June 9. In order to enter these dates in the chart, April 7 was transposed to 34 days before calving; April 14, to 27 days before calving; April 21, to 20 days before calving; April 28, to 13 days before; May 5, to 6 days before; May 12, to 2 days after calving; May 19, to 9 days after; May 26, to 16 days after; June 2, to 23 days after; and June 9, to 30 days after. The counts made on each cow were recorded in this same manner for each parturient period. The counts made on the day of calving, ten counts on nine different cows, were averaged to give a value representing the level of the blood cells on that particular day with reference to parturition. Likewise, on the first day after calving the blood of five cows was examined. An average of these observations represents the average level of the blood cells on this day in the parturient period. The variations from day to day for the cellular components of the blood are recorded in figure 1 for the period extending from the thirty-fourth day preceding calving to the thirty-fourth day following calving. For example, on the day of calving ten counts on nine different cows gave an average of 6.23 million erythrocytes per cubic millimeter, and the first day after calving five observations on five different cows gave an average of 6.28 million erythrocytes per cubic millimeter.

The same procedure was used in arranging the data on the "infected" group for presentation in figure 2.

RESULTS

A comparison of figures 1 and 2 indicates no marked differences between the two sets of data, *i.e.*, infection with *Br. abortus* produced no definite trends in the blood picture during the parturient period which were not also noted at a comparable period in the same animals previous to infection. (A report on the effect of Bang's disease on the blood picture during the entire period of infection will be published later.) On the other hand, definite similarities existed in these two groups in the general trends of change for the different types of cells studied.

As can be seen in figures 1 and 2, the most noticeable changes in the blood picture occurred during the ten-day period immediately following calving. The post-parturient period was, therefore, divided into (a) the ten-day interval immediately following calving and (b) the succeeding 24-day period. The averages of the total and differential counts of the cells for the pre-calving and the two subdivisions of the post-calving period, for both "normal" and "infected" groups, are given in table I. A comparison can readily be made in either group between these averages for the dif-

TABLE I

	"Normal" Group of Animals			"Infected" Group of Animals		
	Pre-Calving Period 34 Days	Post-Calving Period		Pre-Calving Period 34 Days	Post-Calving Period	
		First 10 Days	Subsequent 24 Days		First 10 Days	Subsequent 24 Days
Erythrocytes (millions per cmm.)	6.092	6.271	5.881*	5.812	5.987	5.422*
Leucocytes (per cmm.)	9168*	7945	8646	6960*	6111	7533*
Neutrophiles (per cent)	37.53	35.8	37.64	37.25	35.30	39.57
Monocytes (per cent)	8.96*	14.07	8.91*	10.06*	15.22	9.59*
Lymphocytes (per cent)	38.22	39.89	38.60	38.55	38.76	35.75
Eosinophiles (per cent)	13.98*	8.76	13.58*	13.19	9.98	14.49
Basophiles (per cent)	0.52	0.47	0.36	0.69	0.52	0.46

*Differs significantly from the average of the ten-day interval of the post-calving period. In each group and for each type of cell the average of the pre-calving period and the average of the last 24 days of the post-calving period were compared with the average of the ten-day period immediately following calving. A mean difference of more than three times the standard error of difference was considered significant.

ferent intervals in the parturient period. The value for the ten-day period immediately following calving was compared statistically with the average value of the pre-calving period and to the average of the last 24 days of the post-calving period for each of the types of cells. The differences which are statistically significant are noted in table I. A mean difference of more than three times the standard error of the difference was considered significant.

The erythrocytes in both the "normal" and "infected" groups showed a slight increase in number in the first five days following parturition (figs. 1 and 2). This apparent increase was followed by a decrease to an average level below that of the pre-calving period. Thus, in the "normal" group the average value for the red cell count increased from 6.092 million per cubic millimeter in the pre-calving period to 6.271 million in the first part of the post-calving period (table I). The average value in the last 24 days of the post-parturient period was 5.881 million. This decrease in the second division of the post-calving period, as compared with the first division, is statistically significant. The erythrocytes of the "infected" group showed changes in the same direction and of about the same magnitude, the values for the three periods being 5.812, 5.987 and 5.422 million per cubic millimeter, respectively. As in the "normal" group, the decrease from the ten-day period immediately following calving to the subsequent 24-day period is statistically significant.

The variation in the average number of leucocytes from day to day is rather great, particularly in the normal group, as is shown in figures 1 and 2. The general trend of the changes is, however, similar in both groups. Thus, the "normal" group showed a progressive change from an average number of leucocytes per cubic millimeter of 9,168 for the pre-calving period to 7,945 in the ten-day period, to 8,646 in the 24-day interval of the post-calving period. The decrease from the pre-calving period to the first ten days of the post-calving period (9,168 to 7,945) is statistically significant. The average values for the "infected" group were 6,960, 6,111 and 7,533 for the respective periods. The decrease from 6,960 to 6,111 and the increase from 6,111 to 7,533 are statistically important. The changes in both the "normal" and "infected" groups are quite similar in that in both the leucocytes decreased immediately after calving and after about ten days increased approximately to the level of the pre-calving period.

The average percentage of polymorphonuclear neutrophiles was quite variable from day to day in both the "normal" and "infected" groups, as given in figures 1 and 2. Because of this marked fluctuation a definite interpretation could not be made. From a consideration of both the figures and table I, however, it is evident that there is a general trend toward a decrease in the percentage of neutrophiles in the ten-day period following calving, with an increase in the latter part of the post-calving period. These changes were not significant in either group.

Wide fluctuations also appeared in the daily averages in the percentage of lymphocytes (figs. 1 and 2), but these changes seem to have been inversely proportional to those of the neutrophiles. As noted in table I, the changes in both the "normal" and "infected" groups were slight, but the trend is toward an increase in the ten-day interval after calving, with a return to about the pre-calving level in the latter part of the post-parturient period. None of the changes is statistically significant.

The percentage of monocytes increased definitely in the first few days after calving in the "normal" group, as shown in figure 1. The averages given in table I adequately indicate this change. The average percentage increased from 8.96 in the pre-calving period to 14.07 in the ten-day period following calving and then decreased to 8.91 in the 24-day period. These changes are both statistically significant. The curve in figure 2 indicates that the daily changes in the percentage of monocytes in the "infected" group were quite similar to those in the "normal" group. The average percentages for these individuals, as shown in table I, are 10.06, 15.22 and 9.59, for the respective periods. The increase from the pre-calving period to the ten-day period following calv-

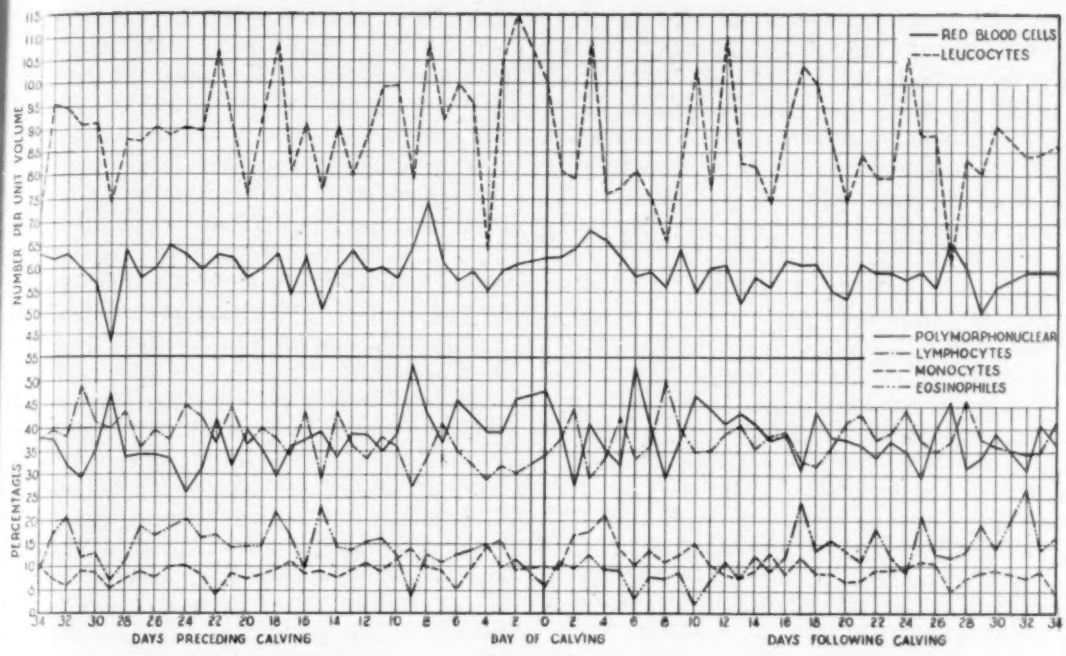

Fig. 1. The average daily level of total erythrocytes and leucocytes per cmm. and the percentages of neutrophiles, lymphocytes, monocytes and eosinophiles, compiled from weekly observations on the "normal" group of cows, covering a period from 34 days before to 34 days after parturition.

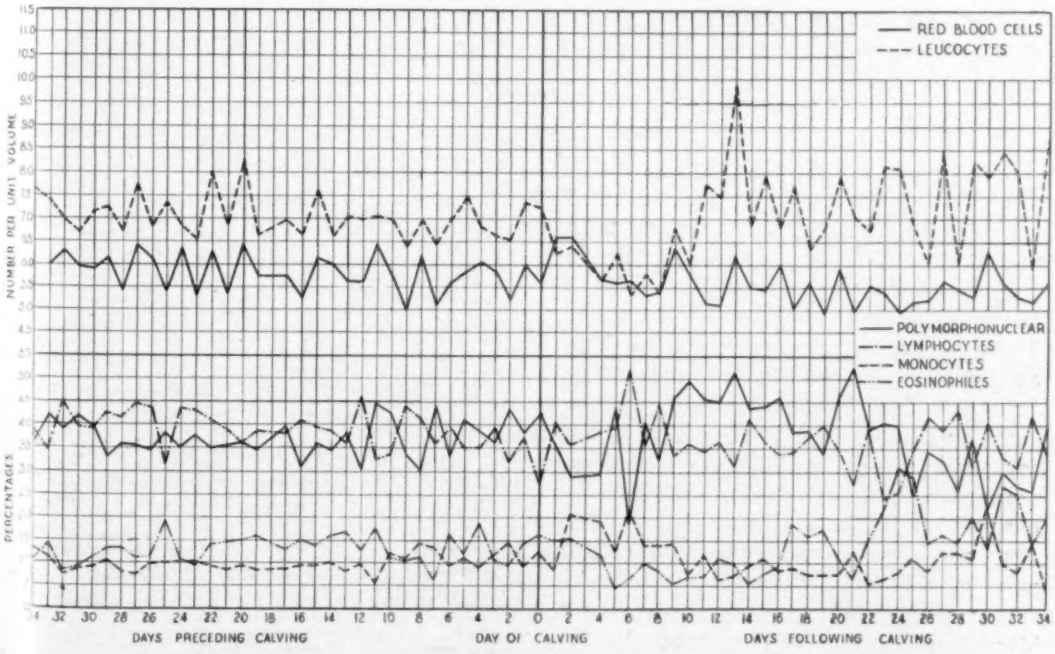

Fig. 2. The average daily level of total erythrocytes and leucocytes per cmm. and the percentages of neutrophiles, lymphocytes, monocytes and eosinophiles, compiled from weekly observations on the "infected" group of cows, covering a period from 34 days before to 34 days after parturition.

ing, 10.06 to 15.22, and also the decrease from the ten-day period to the latter 24 days of the post-calving period, 15.22 to 9.59, are statistically significant.

The average percentage of eosinophiles is approximately inversely proportional to the percentage of monocytes, as may be seen in both figures 1 and 2. The trend is quite similar in both the "normal" and "infected" groups, showing a decrease in the period immediately following calving and then an increase to about the level of the pre-calving period. In the "normal" group the decrease from 13.98 to 8.76 and the increase to 13.58 in the latter part of the post-calving period are statistically significant. Comparable values were obtained in the "infected" group, in which the decrease from 13.19 to 9.98 and the increase to 14.49 are just below the level of statistical significance (2½ times the standard error of the difference).

Because of the small number of basophiles in cattle blood (less than 1 per cent, on an average) no attempt was made in figures 1 and 2 to show their daily variations. The average percentages for the different periods, as listed in table I, indicate a lack of any significant changes in this type of leucocyte during the parturient period.

DISCUSSION

The daily averages given in figures 1 and 2 are representative of the general trend of the variation during the parturient periods of 24 cows. The blood pictures of the individual cows were comparable with the general trends as given in figures 1 and 2. Those which deviated from the herd averages may have resulted, at least in part, from the fact that the weekly counts on these particular cows did not give a representative picture of all of the changes occurring in the parturient period. This explanation is based upon daily observations made on one of the cows throughout most of the infected parturient period.

A summary of previous reports on the blood counts of cattle was made by Scarborough[3] in 1931. Most of the data studied consisted of isolated counts on different individuals. This author concluded that pregnancy produced no change in the total leucocyte number or in the percentages of the different types of leucocytes. The total number of erythrocytes per unit volume was somewhat lower in the pregnant than in the nonpregnant animals. The observations reported in this paper are limited to a period extending from 34 days before to 34 days after parturition and, therefore, are not comparable with the data reported by Scarborough.

Since in both groups the total number of erythrocytes per cubic millimeter increased, although not significantly, above the pre-calving levels, then decreased significantly to slightly below the pre-calving level, it is concluded that this trend is of some biologic importance. Possibly, this increase which occurs during the few days immediately following calving in both the "normal" and "infected" groups may be explained by the changes in the peripheral blood volume as a result of the involution of the uterus. This process is accompanied by a reduced supply of blood to this organ; hence, it is logical to assume that the extra blood supply is distributed in the peripheral system with a corresponding increase in the erythrocytes per unit volume. The vascular changes and the cellular activity during the process of uterine involution have been described by Hallman[4] and Uren.[5]

There was little change of significance in the total number of leucocytes per cubic millimeter until the period immediately following parturition. The rather marked decrease in the ten-day period subsequent to calving, followed by a return to about the pre-calving level, is probably a result of the requirement of the leucocytes in the uterus during involution and in the udder during the initiation of lactation. It may be of significance that in this period the

[3]Scarborough, R. A.: The blood picture of normal laboratory animals. Yale J. Biol. & Med., iv (1931-32), p. 69.

[4]Hallman, E. T.: Further studies in the diseases of the reproductive organs of cattle. Cornell Vet., xiv (1924), p. 254.

[5]Uren, A. W.: Involution of the uterine mucosa in the ewe. Michigan Agr. Exp. Sta. Tech. Bul. 144 (1935).

proportions of neutrophiles and eosinophiles, the phagocytic cells, were reduced in the peripheral blood. The decrease in the percentage of neutrophiles is not statistically significant in either the "normal" or "infected" group, but the change in both groups was in the same direction and of about the same magnitude so that it might be biologically important.

The most marked and most consistent changes in the proportion of the different types of leucocytes occurred in the percentages of monocytes and eosinophiles. During the ten-day period immediately following calving the percentage of monocytes increased considerably while that of the eosinophiles decreased about the same amount. The inverse relation which seems to exist between these two cellular types would suggest the presence of a negative correlation, either as a result of the response to the stimulus produced by parturition or as a result of the difference in their rate of removal from the peripheral circulation.

SUMMARY

The daily averages of the various cellular constituents of bovine blood during a 34-day period preceding and a 34-day period following parturition are presented in graphic form.

Infection with *Brucella abortus* does not greatly influence the blood count during the parturient period, since the general trends, apparent in both "normal" and "infected" cattle, were similar.

The number of erythrocytes per cubic millimeter increased slightly immediately following calving and then decreased somewhat below the pre-calving level in the last 24 days of the post-calving period.

The total number of leucocytes decreased significantly immediately following parturition and later increased to about the pre-calving level.

There were no significant changes in the percentages of either the neutrophiles or the lymphocytes in the period studied.

The percentage of monocytes increased considerably in the ten-day period following calving, but returned to the normal or pre-calving figure in the following period.

The percentage of eosinophiles seemed to vary inversely with that of the monocytes, since in the ten-day period following parturition the proportion was significantly decreased after which there was a return to about the pre-calving level.

The fluctuations which normally occur in the number of leucocytes and in the proportions of the different types are greater in the parturient period than at other times. Therefore, results from a single blood count made on a cow during this period of physiologic disturbance should not be confused with a pathologic response.

Arteriosclerosis in Cattle Associated with Pulmonary Ossification*

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ARTERIOSCLEROSIS IS A common disease of man which is frequently associated with senility. In animals, however, this condition is more rarely encountered and is usually recognized only at autopsy. Arteriosclerosis has been reported in different species of domestic animals from time to time, including cattle, horses, sheep, dogs and fowls, but few investigational studies have been made of this condition in large animals and literature on the subject is meager.

In this paper it is the intention to consider somewhat briefly arteriosclerosis as it occurs in cattle, to give a general picture of the gross lesions and histopathology as seen in cases that have come under our observation over a period of years and to report an unusually interesting case that was received recently at this laboratory.

Some observations with regard to arteriosclerosis in animals have been made by European investigators. Among these may be mentioned Lyding[1] and Kitt,[2] who described the lesions, particularly in cattle. Sivori[3] and Lignières[4] made early studies of arteriosclerosis in cattle and sheep in Argentina, and the latter investigator reported what he considered an epizoötic form of the disease in sheep.

INCIDENCE

There are very few published data concerning the incidence of arteriosclerosis in cattle in the United States. Goldberg[5] reported a number of cases in different species, three of which were in cattle. We have been unable to find other published reports of detailed studies made of the disease in animals of this country. Practically all of the cases of arteriosclerosis in cattle that have come to our attention were those encountered by federal inspectors at officially inspected slaughtering establishments on routine meat inspection and were submitted to our several pathologic laboratories for histopathologic studies. As far as we have been able to ascertain from our early records, the total number of cases of arteriosclerosis received at our laboratories over a period of some 25 years has not exceeded 60. A large proportion of these cases were examined at our branch laboratory at Chicago, Ill.[6] A limited number of cases also have been reported from our branch laboratory at Denver, Colo.[7]

As a rule, only the well-marked cases of arteriosclerosis are submitted for laboratory examination. Consequently, the number of such cases received at our laboratories does not necessarily indicate the total number encountered on meat inspection. It points, however, to the probability that such vascular lesions of cattle are comparatively rare in this country. Incidentally, it may be mentioned here that very few cases of arteriosclerosis in either sheep or swine have been received at our laboratories.

ETIOLOGY

We have little specific information concerning the cause of arteriosclerosis in cattle or other animals. Hutyra and Marek[8] state that although little is posi-

*From the Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture.

[1]Lyding, H.: Zur Kenntniss der Arteriosclerose bei Haustieren. Zeit. f. Tiermed., xi (1907), p. 359.

[2]Kitt, Th.: Path. Anat. d. Haustiere, ii, p. 407.

[3]Sivori, F.: La mancha in sheep. Rev. Gén. Méd. Vét., xix, No. 221, pp. 237-256.

[4]Lignières, J.: Arteriosclerosis epidemica in el ovina. Rev. Zootècnica, iv (Aug. 1912); trans. by F. Torrance: Am. Vet. Rev., xliv (Dec. 1912), pp. 284-292.

[5]Goldberg, S. A.: Arteriosclerosis in domestic animals. Rpt., New York State Vet. Coll., 1924-1925 (1926), pp. 163-172.

[6]Data submitted by Dr. J. S. Bengston, Chicago branch pathological laboratory, U. S. Bureau of Animal Industry.

[7]Case reports by Dr. C. L. Davis, Denver branch pathological laboratory, U. S. Bureau of Animal Industry.

[8]Hutyra, F., and Marek, J.: Pathology and Therapeutics of the Diseases of Domestic Animals (1926), ii, pp. 820-835.

tively known concerning the cause of this condition in animals, it is their opinion that vascular degenerative changes may result from the action of toxins, and that autotoxins and injurious waste products under the influence of radical changes in nutrition probably play an important rôle. Lignières[4] was of the opinion that the cases he studied in cattle were of bacterial origin. Some investigators are of the opinion that such vascular lesions in animals are due to derangement of calcium metabolism. In this connection, it is of interest to note some of the suggested causes of arterioscle-

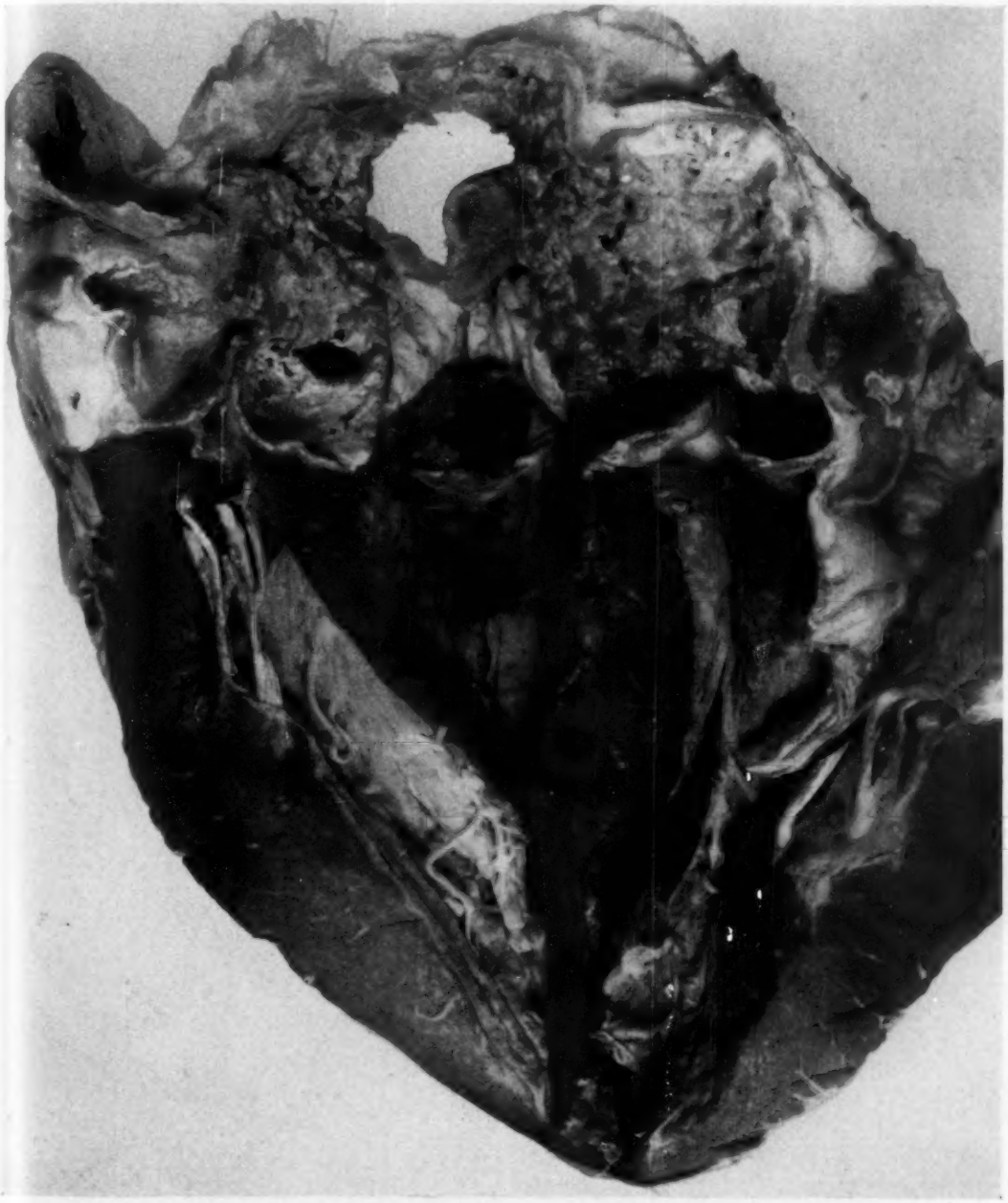


Fig. 1. Heart of heifer. Note extreme calcification of auricles, valves and other structures involved, including chordae tendineae.

Fig. 2. Portion of aorta of heifer, showing several branches. Note marked thickening and calcification, and corrugated appearance of the intima.

rosis in man, such as increased functional demands, nutritional and metabolic disturbances, and toxic influences, either bacterial or chemical. Eventually it may be found that arteriosclerosis in animals is due to similar causative factors. In regard to the matter of age as a possible factor in the causation of such lesions in cattle, our case records show that the number of affected animals in which the ages were from 2 to 4 years greatly exceeded those ranging in age from 5 to 10 years or older, the ratio being almost 3 to 1. As a specific example, the case to be reported here was only 1½ years of age. Thus, it would appear evident that age is of minor significance in the incidence of arteriosclerosis in cattle.

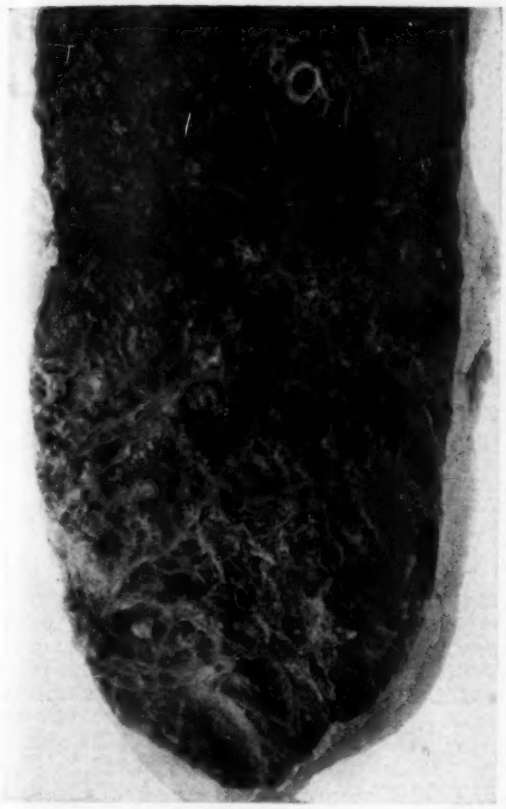
GROSS LESIONS AND HISTOPATHOLOGY

It seems to be the consensus that the lesions of arteriosclerosis are primarily a degenerative process. Due to circulatory disturbance and continued overdistention, there is vascular weakening, and the subsequent proliferative changes in the intima are largely compensatory in character, or an attempt to retain the normal caliber of the vessels. Eventually the proliferative changes involve the entire vessel wall and may be followed by the degenerative processes, necrosis and calcification.

In these cases of arteriosclerosis in cattle studied, we have found considerable variation in the character and extent of the lesions in different animals. In one group of cases there were well-marked lesions of the aorta and its larger branches, with rather frequent heart involvement. Only rarely was there evidence of generalized lesions in these cases.

In another group of cases there was more of a generalization or extensive involvement of the peripheral arteries and arterioles. In these cases the greatly thickened vessels were seen in the gross specimens as small, whitish, nodular-like lesions, particularly on the cut surface of

Fig. 3. Portion of voluntary muscle of heifer in which several of the enlarged, rigid and partially calcified arteries can be seen.



Upper left: Gross section of lung of heifer. Note extensive ossification, giving the lung tissue a spongy appearance. The ossification is more pronounced in the lower portion of the photograph.

Lower left: Section of wall of aorta of heifer, illustrating the extensive atheromatous changes involving both the intima and media in this particular area (x 72).

Lower right: Section of wall of aorta of heifer, illustrating the more advanced changes of necrosis, hyalinization and calcification. Note partially necrotic fragment of media surrounded by the necrotic and calcareous debris (x 72).

Fig. 4

the skeletal musculature. Occasionally such lesions have been suspected of being parasitic. In some of these cases the vascular thickenings also may be observed grossly in the different organs, as in the kidney, liver and spleen. Our findings in this type of arteriosclerosis are contrary to those of Hutyra and Marek,[8] who state that chronic arteritis in animals is confined to the large arterial trunks.

In those cases in which there is an associated ossification of the lungs, these organs have a spongy appearance, particularly on the cut surface, and which on pressure give a crackling or crunching sound due to breaking of the numerous small spicules of bone distributed through

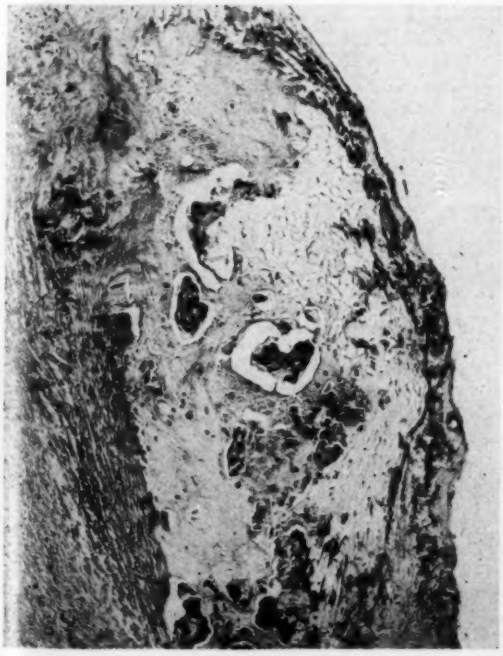


Fig. 5

Fig. 6

Fig. 7. Section of wall of aorta of heifer, illustrating one of the marked prominences extending into the lumen. There was practically complete destruction of the intima and media in this area, and early ossification may be seen in the fibrous portion of the lesions (x 72).

the lung substance. Ossification of the lung tissue has been observed in about 25 per cent of the cases received at our laboratories.

The histopathology, as found in the first group of cases described in which the aorta and its branches were affected, usually consisted of marked thickening of the arterial walls, with the usual atheromatous changes consisting of hyalinization, fatty changes, necrosis, calcification, extensive fibrous proliferation in some areas, and in some cases formation of bony cellular elements. The vascular lesions described in the accompanying case report are representative of an extreme case of this type of arteriosclerosis in cattle.

In the group of cases in which the lesions were generalized, including the peripheral vessels, the lesions consisted chiefly of vascular thickening due to the fibrous proliferation, and usually in these cases there was little evidence of calcification. As a result of the extreme fibrous proliferations in these cases, there is complete occlusion of the lumen of many of the small arteries, and numbers of newly formed vessels may be seen in the proliferated connective tissue immediately surrounding the occluded vessels.

The description given of the ossified lung lesions in the accompanying case report may be considered as characteristic of the histopathology of the usual case of ossification of the pulmonary tissue of cattle, as such lesions have been observed in the different cases.

REPORT OF A CASE

The case of arteriosclerosis reported here and described somewhat more in detail was observed on autopsy at an official slaughtering establishment at Oklahoma City, Okla., and forwarded to this laboratory by Dr. J. H. Kitzhofer, inspector in charge. The tissues submitted, consisting of heart, aorta and its branches, lungs, liver, spleen and portions of the musculature, were from a heifer 1½ years of age. In addition to the usual description of the gross lesions in the tissues submitted, Dr. Kitzhofer made the following additional comment

Fig. 8. Portion of wall of aorta of heifer in which the more extreme destructive changes can be seen. Note extensive disintegration, with cavitations in some areas. Fibrosis and early osseous changes may be seen in this section (x 72).

concerning his observations in this case: "We found the entire arterial system heavily calcified, the intima of all arteries having thick coats of brittle deposit. There were also deposits of calcified material along the esophagus and various tendons, especially at the insertions close to the bone. The animal was in poor condition bordering on emaciation."

Gross Lesions.—Extensive calcareous deposits could be seen on the inner surface of the auricles and around the origins of the aorta and other large vessels, giving the endocardium and intima of the vessels a roughened or corrugated appearance. The calcareous changes also involved the heart valves and chordae tendineae (fig. 1). The calcareous deposits, or plaques, also could be seen extending along the intima of the aorta, its branches, and also the pulmonary artery (fig. 2). The larger arteries of the musculature were apparently enlarged, very prominent and quite rigid (fig. 3). The lungs did not collapse and portions of the surface had a roughened or nodular appearance. On pressure there was a crackling or crunching sound and on gross sectioning, a spongy appearance (fig. 4).

Histopathology.—The lesions of the aortic wall varied somewhat in degree of involvement in different portions of the vessel. Sections from different portions of the wall showed thickened and raised areas in which could be seen early atheromatous changes consisting of hyalinization, mucoid and fatty changes, granular disintegration and calcification (fig. 5). In other areas there were more extensive necrotic changes and calcification, and occasional remnants of the partially necrotic media could be seen (fig. 6). In those areas in which the more prominent sclerotic lumps extended well into the lumen, the intima had largely disappeared and there were marked destructive changes in the media (fig. 7). In some portions extensive disintegration and cavitations were seen. Occasionally in these same sections could be seen areas of extreme fibrosis, calcification and bone-cell formation, or early ossification (fig. 8). Changes similar to those described in the aorta also were seen in the coronary artery and its branches.

The osseous changes in the lungs were rather uniform, the numerous small bony deposits being diffusely distributed throughout the interlobular connective tissue. It appeared evident that an extensive proliferation of the interstitial tissue had preceded the bone metaplasia (fig. 9). Calcareous deposits were numerous, and the osseous changes were seen as small, bony plates and spicules occurring in the small, fibrosed areas of interlobular tissue (fig. 10). Occasional small areas of practically normal lung tissue were observed between the partially ossified areas. There was well-marked

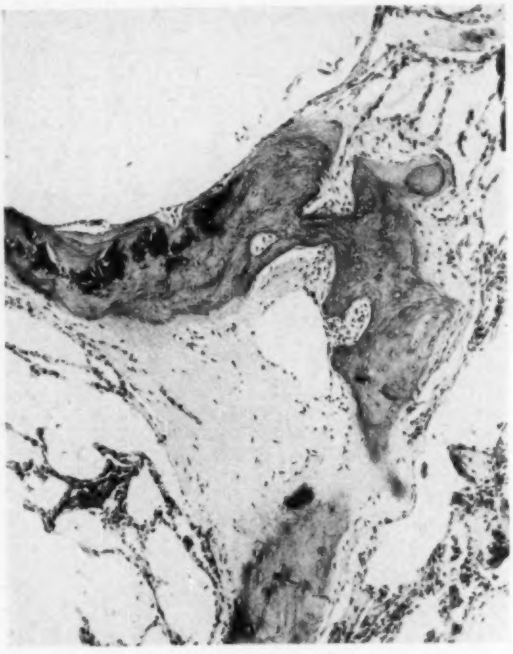


Fig. 9. Section of lung of heifer in which the several changes, comprising fibrous proliferation, calcification and ossification, are all present in a single area (x 72).

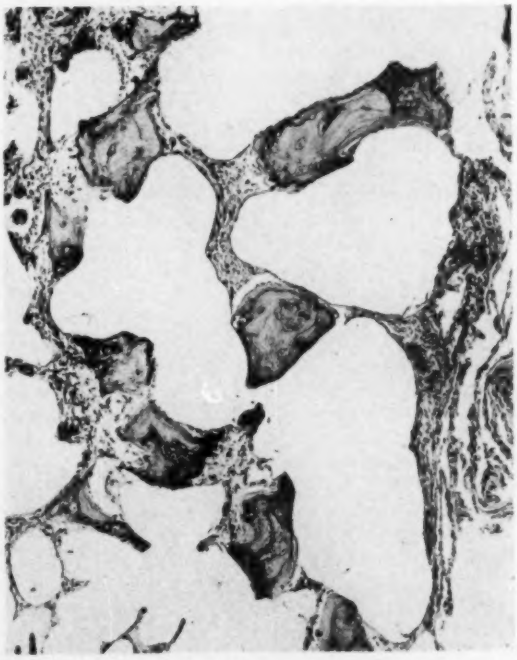


Fig. 10. Section of ossified lung tissue of heifer in which can be seen numbers of the bony plates in the proliferated interstitial tissue (x 72).

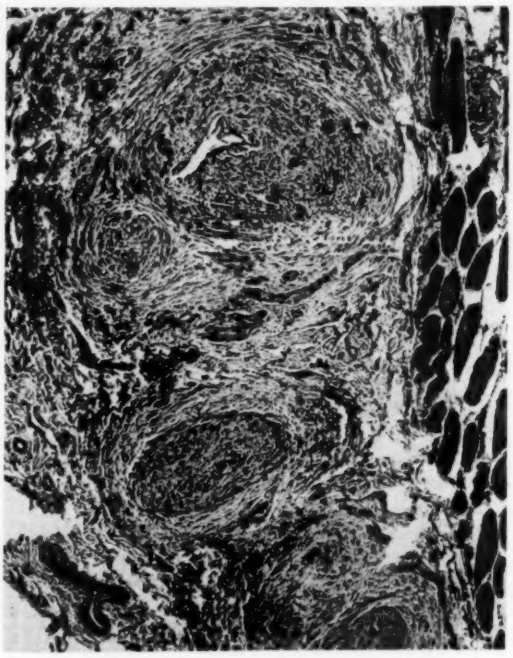


Fig. 11. Section of skeletal muscle of bovine animal, illustrating the marked vascular thickening, and complete occlusion of the lumen, of a number of small arteries and arterioles, as observed in cases of peripheral arteriosclerosis in cattle (x 72).

sclerosis of the arteries of the lungs and also of the liver and spleen. The lesions as described in the peripheral type of arteriosclerosis are illustrated in figure 11.

SUMMARY

Brief consideration is given to previous observations of investigators relative to arteriosclerosis in cattle and brief reference is made to the etiology and incidence of the disease in the bovine species.

A general description is given of the gross lesions and histopathology of arteriosclerosis in cattle and associated ossification of the pulmonary tissue, as observed in a series of cases, and an unusual case is reported in a heifer 1½ years of age.

Little is known concerning the etiology of arteriosclerosis in cattle and other animals. One interesting observation made in connection with these studies would seem to definitely indicate that arteriosclerosis occurs more frequently in comparatively young cattle, or at least in animals which could not be considered as aged. This finding would tend to eliminate age as an important factor in the etiology of this condition in cattle.

The observations made with regard to cases of vascular lesions in cattle encountered in the federal meat-inspection service over a considerable period of years definitely indicate that arteriosclerosis is not of common occurrence in the bovine species in this country.

Iodized Mineral Oil as a Treatment for Bovine Mastitis*

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EFFECTIVE GERMICIDAL treatment for bacterial infection of the bovine udder to be given directly, by injection through the teat canal, would be of great practical value in combating mastitis. For practical use under field conditions the preparation injected would have to be germicidal against the common microörganisms encountered including species of streptococci, staphylococci, micrococci and bacilli; effective in a few applications; relatively nontoxic to the epithelial cells; noncoagulant for the udder secretion; and applicable without elaborate laboratory procedures.

The purpose of this report is to call attention to preliminary experimental work done at the Florida Agricultural Experiment Station on the therapeutic value of iodized mineral oil as a treatment for udder infections.

The reputation of iodine as a germicide has long been established. But iodine, like the other halogens, chlorine and bromine, is a highly active element, combining not only with the proteins of bacteria in the destruction of the bacterial cells, but also with the proteins of animal tissues. When used in too high concentrations iodine may seriously damages the tissues.[1] Salle and his coworkers,[2] however, tested the germicidal properties of iodine against *Staphylococcus aureus* and *Eberthella typhosa* and correlated the effectiveness of 15 germicides against these organisms with their toxicity for living embryonic chick-heart tissue. Iodine in concentrations of 1:3,520 and 1:3,370 destroyed these bacteria within ten minutes, while with the same length of exposure it required a concentration of 1:650 to stop the growth of the embryonic chick-heart tissue. These results indicate considerable latitude for the use of iodine.

Since it was shown that comparatively weak concentrations of iodine were germicidal and not particularly detrimental to animal tissues, it was thought that this drug, incorporated with an appropriate vehicle, might be valuable for treating chronic mastitis.

MATERIALS AND METHODS

Since highly refined mineral oil is known to possess certain qualities beneficial to inflamed tissues, it was reasonable to believe that it would prove desirable as a medium to give bulk for the administration of the iodine. Accordingly, a dairy herd was selected in which numerous cases of mastitis had developed within the past three years. Animals were examined carefully to determine definitely that chronic mastitis was present. The diagnosis of mastitis was made by the macroscopic appearance of milk from suspected quarters, by the microscopic examination of stained smears prepared from fore milk that had been aseptically drawn and incubated, by culturing the microörganisms on blood agar, by palpation of the udder to detect any physical abnormalities, and upon the general history of the case.

The history of the herd showed that individual animals were affected with mastitis and that during occasional "flare ups" the gross appearance of the milk was altered. Clinical examinations revealed varying degrees of fibrosis in a large number of the quarters. Microscopic and cultural examinations of the fore milk from affected quarters showed a high leucocyte content, and streptococci, staphylococci and micrococci were associated with these alterations.

The solution of iodine in mineral oil was prepared by mixing resublimed iodine U.S.P. crystals in U.S.P. liquid petrolatum (heavy medicinal mineral oil). In the first studies the iodized mineral oil was prepared by adding the iodine crystals to a small amount of oil in a mortar and triturating with a pestle. The undissolved iodine crystals remaining after triturating were allowed to settle in the mortar for a few moments. The iodine solution was decanted, leaving the undissolved portion for further trituration with oil. The trituration and decanting were repeated until the iodine

*From the Florida Agricultural Experiment Station.

[1] Iodine from Scratch to Major Operation. (Iodine Education Bureau, N. Y.)

[2] Salle, A. J., McOmie, W. A., Shechmeister, I. L., and Foord, D. C.: The evaluation of a group of germicides by the tissue culture technique. J. Bact., xxxvii (June 1939), pp. 639-645.

TABLE I—Therapeutic Value of Iodized Mineral Oil in Some Cases of Mastitis

Status of Infection Before Treatment					Amount Iodized Mineral Oil Injected (cc.)	Dilution	Number of Treatments	Organisms Cultured After Treatment	Number of Days Within Which Organisms Disappeared from the Milk	Number of Weekly Examinations Made After Treatment
Cow No.	Quarter Affected	Months Known to Be Infected	Type of Organism Cultured	Type of Infection						
625	LF	8	HS	C	300	1:1,500	1	—	2	7
519	RR	3	NHS	C	200	1:1,000	1	—	1	8
512	LF	4	NHS	C	100	1:1,000	1	—	2	8
165	RR; RF	12	HS	C	225	1:1,000	1	—	7	3
168	LF; RR	12	HS	C	225	1:1,500	1	—	2	3
691	LR	4	Mi	C	200	1:1,500	1	—	1	8
136	RR	4	Staph	C	300	1:1,000	1	—	2	3
312	LF	2	HS	A	250	1:1,500	1	—	6	4
375*	RF	3	NHS	C	150	1:900	1	—	2	8

*Dry cow.
LF: left fore.
RF: right fore.
RR: right rear.
LR: left rear.
C: chronic.
—: acute.
A: none present.
HS: hemolytic streptococci.
NHS: nonhemolytic streptococci.
Mi: micrococci.
Staph: staphylococci.

crystals were practically all dissolved. Small particles of iodine crystals which may have been decanted were dissolved by shaking the iodized mineral oil at occasional intervals. Recent trials showed the iodine was dissolved more readily in the oil if the crystals were first triturated with a small amount of ether. Evaporation of the ether during the trituration left the iodine in a pulverized form which facilitated dissolving upon trituration with oil.

Affected quarters were treated by injecting 100 to 300 cc. of iodized mineral oil directly into the previously emptied milk cistern *via* the teat canal. In an effort to distribute the iodized mineral oil over the epithelial surface of the milk cistern, the quarter was lightly massaged immediately following the injection. When an amount up to 100 cc. was used, the drug was allowed to remain within the udder overnight. Where the injection consisted of more than 100 cc., the treated quarter was stripped thoroughly within 45 minutes, removing as much of the injected material as possible. In most instances the free iodine in the solution injected into the milk cistern was partially decolorized by chemical union with the udder secretion while being distributed over the epithelial surface by massage. The degree of decolorization depended upon the stage of lactation, thoroughness of removing the udder secretion before injection, thoroughness of massage, amount and concentration of free iodine injected and surface area of the tissues treated. While satisfactory results have been obtained from 100-cc. injections in certain cases, larger doses up to 300 cc. seem to be advisable in other cases. However, the amount to be used will vary with the location and extent of the infection. The degree of decolorization of the free iodine secured following massage was since used as an index of the amount to be injected.

Results and Discussion

Twelve lactating cows harboring hemolytic streptococci which produced chronic mastitis in one or more quarters, four cows harboring micrococci in seven quarters and one cow harboring staphylococci in one quarter were successfully treated by injecting iodized mineral oil into the milk cistern.

The injections initiated a temporary inflammatory reaction of the udder tissues with the occurrence of erythrocytes in the fore milk, and diminished milk flow in some instances. No permanent ill effects have been detected as a result of injecting this preparation into the chronically infected lactating quarters. The inflammatory reaction which occurred as a result of the treatment subsided, the appearance of the milk returned to normal and the milk flow was restored within ten days. Mastitis microörganisms have not been detected in microscopic examinations of stained smears prepared from incubated samples of fore milk of a number of quarters receiving the

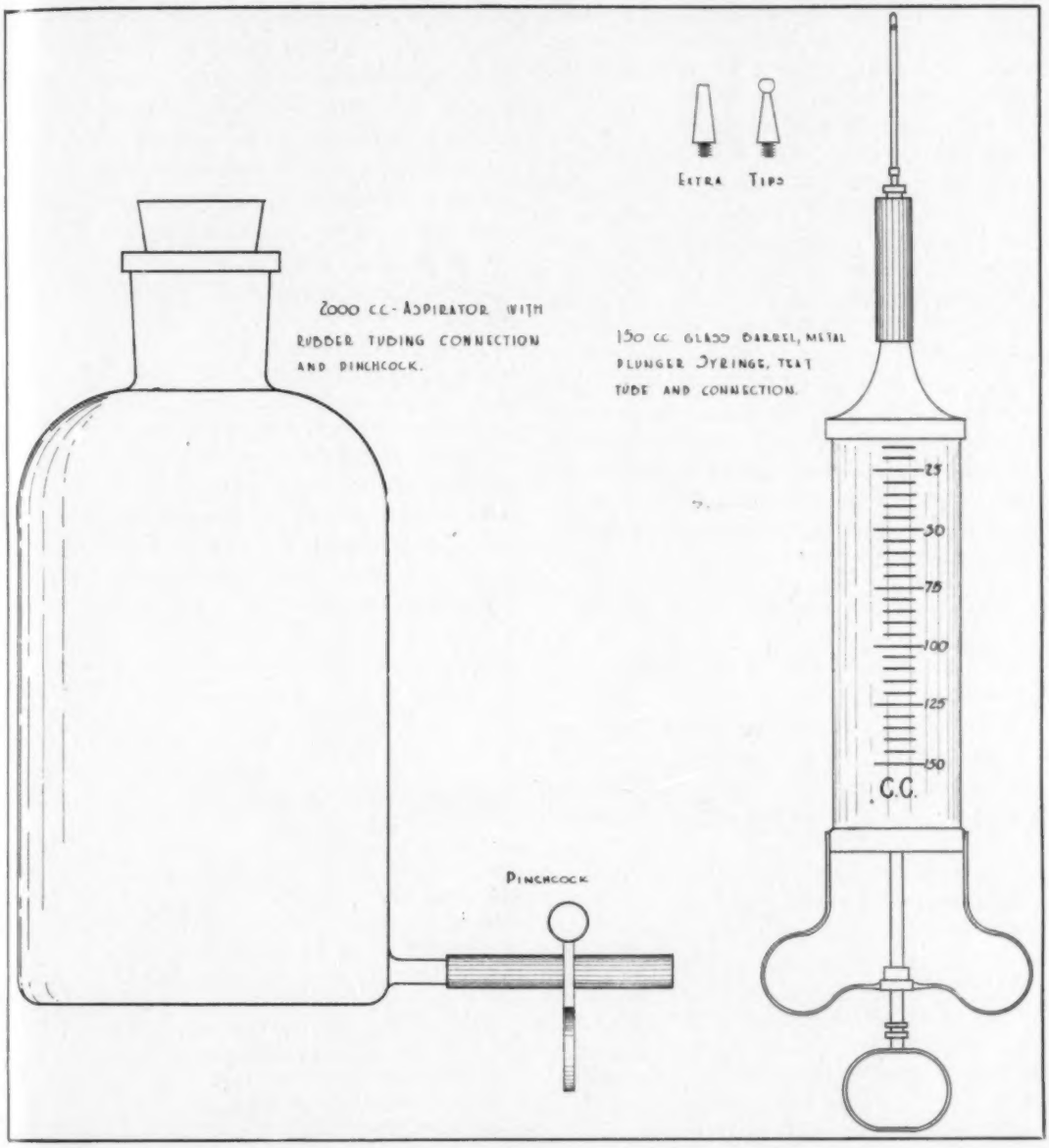

Fig. 1. Apparatus used in injecting iodized mineral oil solution into infected udder quarters via the teat canal.

treatment. These examinations were made at weekly intervals. The longest period of observation was 60 days. One case of acute streptococcic mastitis treated with iodized mineral oil appeared to be aggravated. Since little information is at hand on the treatment of acute mastitis with iodized mineral oil, it would not seem advisable to superimpose this therapeutic inflammation upon an extensive acute inflammation already existing.

While the number of experimentally treated cases is too small to form definite conclusions, the results indicate that chronic udder infections may be successfully treated by injections of iodized mineral-oil solution into the infected quarter *via* the teat duct. Preliminary observa-

tions on two animals indicated that udder infections also may be overcome by injections of iodized mineral oil into the milk cistern *via* the teat duct during the "drying off" period prior to calving. Further experimental work will be necessary to develop the technic best suited to accomplish the desired results. Beneficial results of the treatment appear to depend upon the germicidal effect on the foci of infection, and upon bactericidal substances which accompany the inflammation following the treatment. It may be assumed that infected foci situated in the lower cistern are accessible and offer favorable opportunity for successful treatment. Foci of infection situated in the upper glandular tissues are relatively inaccessible and offer less favorable opportunity for successful treatment.

According to Johnson,[3] the free iodine in mineral oil is gradually decolorized by combining with the casein of milk and that iodine combined with milk casein is no longer as bactericidal as elemental iodine. This author states that skimmed milk will ultimately decolorize about 0.36 per cent of its weight of elemental iodine when the two are actually homogenized together. Thus, it is obviously necessary to milk the quarter before introducing the iodized mineral oil.

[3]Johnson, F. F.: Personal communication.

CONCLUSIONS

Preliminary observations on a limited number of animals indicate that iodized mineral oil has valuable therapeutic properties as a treatment for chronic bacterial infections of the bovine udder. These results justify further experimental work on that treatment for mastitis.

Anatomic Changes in the Bovine Uterus During Pregnancy*

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THE GROSS ANATOMY of the nonpregnant bovine uterus has been described by Sisson-Grossman (1938), Chauveau (1891) and many others. Hilty (1908) published a report of several isolated cases that he observed, in both gross and microscopic detail. Zupp (1924) published a paper on the cyclic variations of the genitalia during the various phases of the estrual cycle. Hummon (1927), Smith (1937) and Chapman and Cassida (1937) made studies of the physiology of reproduction and the changes of the estrual cycle. Dukes (1927) annotated some of the gross changes occurring during pregnancy.

MATERIAL AND METHODS

The material used in this study included the genitalia of 40 pregnant animals. Gross and microscopic examination of these organs showed no evidence of either systemic infection or gross pathologic change.

Portions of the uterine wall of the pregnant and nonpregnant horns and a portion of the uterine end of the cervix of each specimen were obtained for microscopic examination. A section of the fetal membranes, taken in the region of the plaque-like growths on the umbilical cord, also was obtained. Sections of the uterine wall included entire caruncles with the fetal membranes attached as well as segments of the intercaruncular region at several different levels of the uterus. In the few instances in which there were no membranes attached in the nonpregnant horn, the intercaruncular areas at the various levels were used.

Several staining technics were employed routinely in the preparation of the slides. Hematoxylin and eosin, Mallory's triple stain (Guyer, 1938) and iron-hematoxylin (Earle, 1939) were used on practically all of the tissues. Several sections were stained with muci-carmine (Guyer, 1938) and others were stained to demonstrate reticular fibers (Wilder, 1935).

DISCUSSION

The nonpregnant bovine uterus is a bicornual structure with curved or tightly spiraled horns that are attached throughout their length by a muscular mesometrium. In the nonpregnant state the length of the uterine horns may vary considerably. The horns may not be of equal length, particularly if the animal has been pregnant. In general, however, the two horns of the nonpregnant uterus are alike in size and shape.

The blood vessels, upon contacting the uterus at its mesometrial attachment, branch longitudinally and laterally on the uterine horns in several directions. Many of the branches do not dip directly into the muscle layers, but extend for some distance in the subserous tissue before dipping deeper and distributing themselves in the manner described by Bressau and Le-Gall (1936). Animals of one or more pregnancies usually exhibit a more prominent vascular system than virgin heifers.

Data relative to the amount of fetal fluid (table I) are inconclusive. Loss or partial loss of the fluids of many of the larger specimens of this series makes it difficult to draw conclusions regarding the normal amount of fluid present. Wislocki (1935) stated that there is a wide variation in the amount of fluids between individuals and even between litter mates.

The more prominent gross changes occurring in the uterus are listed in table I. The curve for uterine weight corresponds roughly to a curve for similar data compiled by Hammond (1927). The measurements of the length of the horns were taken with a steel tape and variations in the position of the uterus when placed on the floor may account for considerable variation in the length of either the pregnant or non-pregnant horn. (See table I.)

On histologic examination, the epithelium of the nonpregnant uterus appears as

*An abridgment of a thesis submitted to the graduate faculty of Iowa State College in partial fulfilment of requirements for the degree of Master of Science, December 1940.

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TABLE I

Spec. No.	Fixative	Age	Breed	Previous Pregnancies	Length of Gestation	Weight of Uterus (Lb.)	Weight of Fluids (Lb.)	Length of Preg. Horn (In.)	Length of Nonpreg. Horn (In.)
35	Formol-Susa	9 yr.	Jersey	4	21 days	1.75	....	6.50	6.50
12	Susa	18-24 m.	Hereford	None	5 wks.	....	....	.....	.....
19	Susa	18-24 m.	Angus	None	5 wks.	1.75	....	6.75	5.50
27	Formol-Susa	18-24 m.	Angus	None	6 wks.	2.25	1.25	10.50	9.00
44	Formol-Susa	3 yr. 4 m.	Jersey	1	43 days	3.00	....	8.50	6.50
36	Formol-Susa	3 yr. 10 m.	Jersey	2	47 days	5.00	2.00	16.00	10.00
23	Susa	?	Shorthorn	Several	7 wks.	2.50	1.00	10.50	8.00
4	Bouin	14 yr.	Jersey	5	61 days	3.00	....	14.00	9.00
21	Formol-Susa	18-24 m.	Hereford	None	9 wks.	2.50	1.25	12.00	8.00
25	Susa	?	Jersey	Several	9 wks.	2.75	1.00	11.00	10.00
15	Susa	18-24 m.	Angus	None	10 wks.	2.75	....	.....	.....
17	Susa	18-24 m.	Angus	None	10 wks.	2.50	0.50	14.00	9.50
24	Formol-Susa	?	Jersey	Several	11 wks.	3.50	1.25	15.50	10.00
9	Bouin	2 yr. 8 m.	Jersey	1	79 days	3.50	....	18.00	11.00
34	Susa	3 yr. 8 m.	Jersey	2	82 days	6.00	2.25	13.00	9.00
29	Formol-Susa	18-24 m.	Hereford	None	12 wks.	4.50	1.75	16.00	9.00
18	Susa	18-24 m.	Hereford	None	12 wks.	4.00	0.75	14.50	9.00
22	Susa	?	Shorthorn	Several	12 wks.	4.25	1.12	18.00	9.50
1	Bouin	?	Jersey	Several	90 days	4.75	1.50	.....	.....
28	Formol-Susa	6-8 yr.	Shorthorn	Several	13 wks.	5.00	3.00	18.00	8.00
37	Formol-Susa	18-24 m.	Angus	None	13 wks.	8.00	2.50	20.00	5.50
3	Bouin	3 yr.	Jersey	1	95 days	12.50	....	47.00	8.50
6	Formol	2 yr. 6 m.	Jersey	None	100 days	....	....	39.50	9.00
20	Susa	18-24 m.	Hereford	None	14 wks.	10.00	5.00	20.00	10.00
30	Formol-Susa	?	Guernsey	Several	14 wks.	8.00	2.00	21.50	10.50
39	Formol-Susa	18-24 m.	Shorthorn	None	14 wks.	8.00	2.00	19.00	.....
40	Formol-Susa	18-24 m.	Angus	None	14 wks.	8.50	2.00	20.00	9.00
33	Formol-Susa	3 yr.	Jersey	1	102 days	9.00	4.00	17.00	11.00
45	Formol-Susa	3 yr.	Jersey	1	102 days	....	....	24.50	12.00
14	Susa	18-24 m.	Hereford	None	15 wks.	9.00	2.00	24.00	7.00
2	Bouin	3 yr. 8 m.	Jersey	1	117 days	11.00	4.00	22.00	8.00
26	Susa	?	Hereford	Several	17 wks.	....	....	28.00	10.00
32	Susa	4 yr. 5 m.	Jersey	3	125 days	20.00	10.00	22.00	13.00
13	Susa	18-24 m.	Hereford	None	17 wks.	16.00	8.00	29.00	13.00
41	Formol-Susa	18-24 m.	Shorthorn	None	18 wks.	....	....	32.00	11.00
11	Susa	18-24 m.	Hereford	None	18 wks.	15.00	4.00	.....	.....
7	Formol	5 yr. 6 m.	Jersey	3	185 days	28.00	6.00	52.00	20.00
31	Susa	3 yr. 10 m.	Jersey	2	205 days	....	....	.....	.....
8	Formol	2 yr.	Jersey	None	260 days	61.00	....	60.00	24.00

a pseudostratified columnar layer that is complete in all regions (fig. 2). Occasionally there are cells that contain droplets of mucin-like material in this layer. Many of the cellular variations described by Bartelmez (1940) as being fixation artefacts also are found.

The intercaruncular epithelium of these specimens shows many variations. In every pregnant specimen of this series there are areas from which the epithelium has disappeared, but there is no specimen in which all of the intercaruncular epithelium has been destroyed. There are always some normal, undisturbed epithelial cells at the mouths of the gland ducts, where they empty into the uterine lumen. The greatest degree of epithelial denudation seems to occur in the middle of the pregnant horn. Chiodi (1926) also found this type of epithelial erosion.

It is of interest to note that the lamina propria or dense connective tissue layer underlying the epithelium does not show evidence of degeneration even though the covering epithelium is completely destroyed (fig. 3). In nearly every eroded region a basement membrane can be demonstrated. This membrane consists of connective tissue in which reticular fibers are very nu-

merous. In many instances capillaries just under the basement membrane become prominent after the epithelium has been destroyed and appear as irregularities on the surface. In some instances, the epithelium appears to be undermined and pushed upward from the basement membrane (fig. 4); this type of change may be a forerunner of epithelial destruction. Epithelial cells that are loosened from their attachment appear vacuolated and show signs of degeneration. In the various pictures seen at the different levels of the uterus, the intercaruncular erosion seems to be a continuous process, beginning early in pregnancy and continuing until late in gestation.

The epithelium of the caruncle presents a much different picture. There is, at all times, a complete, unbroken caruncular epithelium. At the crests of some of the caruncular villi and at the outer margins of some of the caruncles near the attaching pedicle, the epithelium may not be distinct, but a continuous cell layer can be demonstrated. The caruncular epithelium differs markedly from that of the intercaruncular areas (compare figures 1 and 2). It is made up of small, flat or cuboidal cells with a compact, dense nucleus and a small amount of cytoplasm. The size of the epithelial cells of this layer has no correlation with the size of the caruncle on which they are found. The average height of the epithelium of the caruncles of the pregnant horn is 0.015 mm.

As the caruncle develops, the epithelium of the surface appears to dip into the deeper structures, producing pits or cords of cells which are later invaded by chorionic tissue. In the nonpregnant uterus and in the nonpregnant horn of the pregnant uterus a scalloping or regular indipping of the epithelium over the caruncle can be seen (fig. 5). This is probably a forerunner of the crypts of the functional caruncle.

The densely cellular lamina propria is compact and contains many embryonic connective tissue cells and is usually infiltrated with lymphocytes. Scattered, isolated smooth muscle cells, making up a rudimentary muscularis mucosa, are seen in this layer, but none of the sections of this series reveals any relationship between these muscle cells and the tunica muscularis.

Many gland ducts can be seen in the lamina propria of the intercaruncular regions, but none occur subcaruncularly. Except for the gland ducts the cellular components of the lamina propria are the same throughout the entire uterus. The only prominent change occurring in the intercaruncular lamina propria during gestation is a decrease in the thickness of the layer and possibly an increase in the number of blood vessels. Accurate measurement of this tissue was impossible due to the amount of shrinkage caused by fixation.

The epithelium of the gland ducts is very similar to that of the surface. It is a tall columnar or pseudostratified type averaging 0.034 mm. in height. These ducts have been described as a superficial type of gland. Although the lumens of these ducts vary markedly, the average diameter in the pregnant horn is 0.068 mm.

In the subcaruncular lamina propria large arterioles project upward from the submucosa. At the base of the developing caruncle these arterioles divide into capillaries, one capillary extending into each caruncular villus. In the large caruncles, in which the lamina propria makes up most of the attaching pedicles, the capillaries extend in all directions from the pedicle into the villi of the dome-shaped caruncle.

During gestation the caruncles show considerable quantitative change. The caruncle that has not been invaded by chorionic tissue appears as an oval, raised area beneath which there is marked vascular congestion of the lamina propria and submucosa. The caruncles to which no membranes are attached are covered by the same type of epithelium found in the intercaruncular regions. No gland ducts open on the surface of the caruncle, but gland tubules exist in the submucosa. As the caruncle increases in size, the area of attachment, which later becomes a pedicle, remains static in size. The tissues grow upward and outward and produce a convex, mushroom-like structure. The oval outline of the primitive caruncle is retained, however, and the long axis of the caruncle re-

mains transverse to the long axis of the horn.

The internal structure of the large caruncle resembles that of the lamina propria, and as growth continues, the vascular channels increase in size. There is also an increase in the size of the vessels passing up into the caruncular area from the submucosa. Growth of the caruncle and chorionic cotyledons is parallel and there is little change in the size of the cells of the caruncle during gestation. As growth of the caruncle continues, the loose tela submucosa may permit a portion of the intercaruncular region to be pulled upward with the pedicle. In this way many gland openings are pulled up on the pedicle and at the base of the caruncle.

The maternal face of the chorion is covered with a complete layer of epithelium. These cells cover the cotyledonary tufts as they project into the crypts of the caruncles and also cover the intercotyledonary spaces, making the fetal-maternal junction epitheliochorial in type (fig. 1).

The cotyledonary epithelium consists of cells that are quite irregular in size. In general, it is a single layer of cells, but it may be two or more layers thick (fig. 1). There are many binucleate and multinucleate cells in this layer, but a true syncytial structure is lacking. In most cases the outline of the individual cells may be determined.

The structure of the plaque-like growths, occurring on the internal face of the amnion in the region of the umbilical cord, was described at length by Santoni (1936). These structures of gestation are present on all of the specimens of this group that are over 5 weeks old. They first appear as small, raised papillae and become increasingly prominent up to the later stages of gestation, when they begin to disappear. Bernard (1859) concluded that these structures may have a function similar to that of the liver and that their disappearance corresponds to the earliest functioning of the fetal liver. Histologically the structure of these plaques is similar in many ways to the structure of the skin. The basal layer of cells appears to be actively growing cells. The central part is made up of large cells with pale cytoplasm and oval, pycnotic nuclei, and the superficial layer appears cornified.

The submucosa of the uterus consists of a layer of loose connective tissue situated beneath the lamina propria. Many uterine glands are present in this layer. Large vessels also are present, particularly in the subcaruncular regions. There are a few reticular fibers in this tissue, but they are sparse as compared with the lamina propria. Little change in the structure of the submucosa can be observed during the course of gestation. Late in gestation the blood vessels become so prominent that the tissue appears edematous and stains weakly.

The glandular epithelium remains constant in size and type throughout gestation. The average height of the glandular epithelium deep in the submucosa is 0.023 mm. The epithelium is cuboidal with a densely staining oval nuclei. There is no evidence of cilia on these cells. The average diameter of the glandular lumens during gestation is 0.056 mm. in the pregnant horn and 0.049 mm. in the nonpregnant horn. The ducts of the glands deep in the submucosa

CAPTIONS TO ILLUSTRATIONS ON OPPOSITE PAGE

Fig. 1. Fetal-maternal union in the deep part of a caruncle ½ in. long (x 400).

Fig. 2. Intercaruncular mucosa of a 16-week pregnancy, showing no erosion (x 400).

Fig. 3. Intercaruncular mucosa of a 14-week pregnancy, showing complete erosion of the epithelium (x 400).

Fig. 4. Intercaruncular mucosa of a 14-week pregnancy, showing partial erosion and epithelial destruction (x 400).

Fig. 5. A caruncle of the nonpregnant horn of a 3-week pregnancy. No membranes are attached. Note scalloping of mucosa (x 400).

Fig. 6. Early caruncular crypt; same section as figure 5 (x 800).

Fig. 7. Intercaruncular submucosa, showing two types of epithelium of gland (left) and duct (right) and the basement membrane (x 800).

Fig. 8. Subcaruncular lamina propria, showing vascular channels extending into caruncular villi (x 400).

Fig. 1.
Fig. 2.

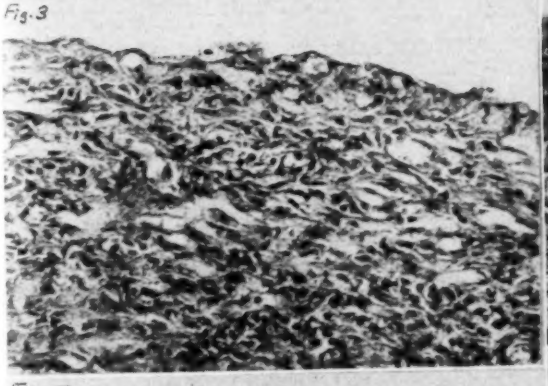
Fig. 3

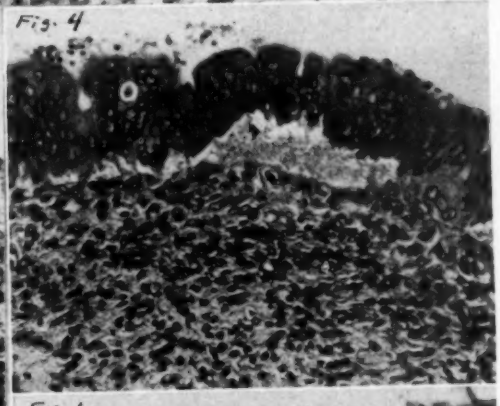
Fig. 4

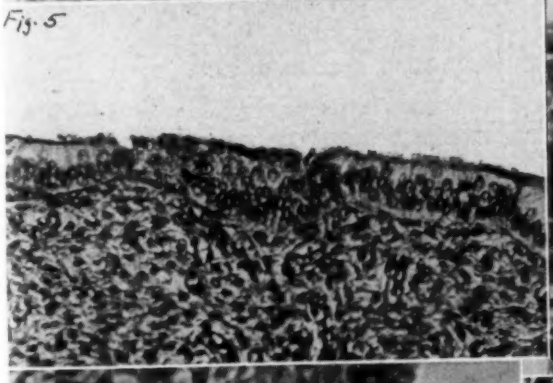
Fig. 5

Fig. 6

Fig. 7

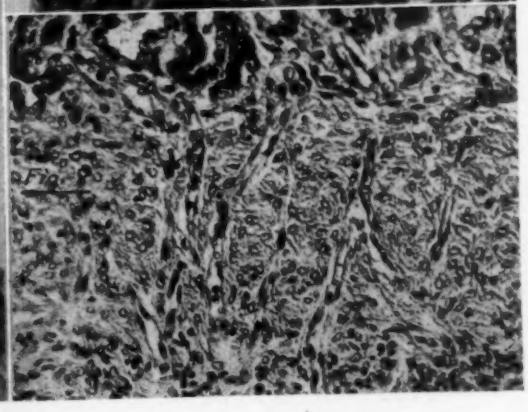
Fig.

differ from the superficial ducts in that the epithelium is lower and more densely staining and the lumens are uniformly smaller.

The myometrium consists of two definite layers of smooth-muscle fibers. The inner layer is circular and the outer one longitudinal. Along the mesometrium, and to a certain extent at the tip of the uterine horns, the two layers blend together and appear more or less continuous. Some of the muscle cells extend upward into the mesometrium. Throughout most of the uterus the larger blood vessels are located between the two muscular layers, but this relation is lost in the mesometrial region and near the tip of the organ. During the early part of gestation in specimens of uteri that have been pregnant previously, the vessels appear as in an area of a mild, chronic inflammation. They are surrounded by a dense layer of connective tissue in which the cellular elements are most numerous at the periphery.

DeBruin (1901) and Allen, Smith and Reynolds (1937) presented evidence to indicate that there was a muscular hyperplasia occurring during gestation. In the specimens considered here, some mitoses of the muscular nuclei were seen in the later stages of gestation. Muscular hyperplasia is not sufficient to prevent a marked decrease in the thickness of the uterine wall. There was no evidence of a syncytial arrangement of the muscle cells in these specimens, although no special technics were employed to study this.

In certain areas along the lateral face of the pregnant horn, during the later stages of gestation, there is only one muscular layer present. Hilty (1908), in discussing this fact, concluded that the longitudinal layers are composed of two bands that become separated as the uterus becomes enlarged. The absence of an outer muscle layer is noted only in advanced pregnancies and only on the lateral face of the pregnant horn.

It is impossible to draw any conclusions from the actual measurements of the myometrium and endometrium made in this study. The primary reason for this is that as soon as the internal pressure of the fluids and fetus is removed, there is a definite contraction of the muscle layers. The reaction of the excised organ is similar to the reaction of the uterus in cesarean section. The muscle layers contract within a few seconds after removal of the fetus. Completely isolated segments of the uterine wall also will contract when placed in a fixing solution, and uteri from which only the fluid has been removed will contract when placed in a solution of 10 per cent formaldehyde.

The serosa, a thin layer of peritoneum, is always present on the external face of the uterus. There is a thin layer of connective tissue, in which there are many fibers and few cells, separating the squamous cells of the peritoneum from the muscle layers of the uterine wall.

SUMMARY AND CONCLUSIONS

Forty specimens of bovine uteri at various stages of gestation were studied. Observations of the many caruncular and intercaruncular sections were made in an attempt to clarify some of the controversial points regarding the anatomy and histology of the uterus during pregnancy. Several conclusions may be drawn:

1) At all times during gestation there is some intercaruncular epithelium on the uterus. This epithelial layer is partially destroyed at all stages between the 21st and 260th days of gestation.

2) Various stages of epithelial destruction may be seen at any time during the periods of gestation studied.

3) The maternal epithelium of the caruncle is complete at all times during gestation.

4) The placentomaternal relationship in the caruncular areas is epitheliochorial.

5) The caruncle develops by an overgrowth of the tissues of the lamina propria pushing the epithelium toward the uterine lumen. The connective tissue is invaded by cords of epithelial cells from the surface which form pockets or canals in the caruncle. These pockets are invaded by and filled with tissues of the chorionic trophoderm.

Observations on the Longevity of the Liver Fluke, Fasciola Gigantica, in Cattle*

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A KNOWLEDGE OF the longevity of the liver fluke in cattle is of practical importance, particularly in establishing proper control methods under field conditions. As far as the writers are aware, no records are available on the length of life of *Fasciola gigantica* in cattle, although the longevity of a closely allied liver fluke, *F. hepatica*, has been reported. Hutyra and Marek[1] state: "The flukes (*F. hepatica*) remain in the liver for a period of 3 to 5 years. Railliet, Moussu and Henry found them after 3 years, and Thomas after five and one-quarter years in animals that had no opportunity to become reinfested during this period. The majority of the flukes, however, leave the liver in from 9 months to 1 year . . ." According to Montgomerie,[2] liver flukes were recovered three years and one month after experimental infection of rabbits with *F. hepatica;* this writer was also of the opinion that *F. hepatica* did not continue to infect cattle for a long period and stated that livers of 2½-year-old cattle commonly show evidence of severe infection without the presence of flukes.

PLAN AND SET-UP OF EXPERIMENT

In January 1937, through the coöperation of Mr. Geo. P. Cooke, president and manager, and Mr. G. P. Judd III, assistant manager, of the Molokai Ranch Ltd., five young steers of weaning age were each given a 1-oz. gelatin capsule containing 800 encysted metacercariae of *F. gigantica*. The cysts were attached to blades of grass, and their viability had been ascertained through experimental infection of guinea pigs receiving cysts from the same lot.

The steers which were used in the experiment were born and had been maintained at all times on the Molokai ranch, located in the central portion of the island of Molokai. This ranch comprises about 40,000 acres of land, most of which is arid, and there are no swamps. The water for the pastures is piped from the high mountains and retained in large reservoir-type tanks from which a larger pipe leads to individual watering troughs equipped with automatic float control. The feed for the animals consists primarily of kiawe beans, salt bush and arid grasses. As far as is known, no liver flukes had ever been discovered in cattle raised on this ranch. As a further check, seven wild deer were killed within the ranch and were found fluke-free.

The plan of the experiment was to examine samples of feces from each steer for fluke eggs every three to six months and to slaughter the animals when fluke eggs were no longer present or were very few in number.

The observations made throughout this experiment are briefly summarized in table I.

DISCUSSION AND CONCLUSION

Fluke eggs were noted in the feces of the animals three months after experimental infection. After one year and four months of infection, a striking decrease was evident in the number of eggs in the feces as compared with previous examination, and the absence of mature flukes in one of the animals (steer 5) slaughtered at that time. By the end of the second year of infection, two steers (2 and 3) completely lost the flukes, but two others (1 and 4) still showed a few fluke eggs in the feces, even to the end of the third year. These two animals still harbored flukes when slaughtered, one three years and one month, and the other three years and four months after infection.

The experiment shows that in an infection with *F. gigantica*, most of the flukes are eliminated by the end of one year, but some of them may survive for at least three years and four months.

(*See table I on next page*)

*From the Hawaii Agricultural Experiment Station. The junior author was associated with the Hawaii station from March 1936 through February 1937; at present he is with the Florida Agricultural Experiment Station, Gainesville, Fla.

[1]Hutyra, F., and Marek, J.: Special Pathology and Therapeutics of the Diseases of Animals (Alexander Eger, Chicago, 1926), ii, p. 471.

[2]Montgomerie, R. F.: On the longevity of *Fasciola hepatica* in experimentally infected rabbits. J. Helminthol., ix (Nov. 1931), pp. 209-212.

TABLE I—Observations on an Experimental Infection of Five Steers with Liver Flukes (Fasciola Gigantica)

DATE	MONTHS FOLLOWING INFECTION	STEER 5	STEER 3	STEER 2	STEER 4	STEER 1
Jan. 12, 1937	..	Fed 800 fluke cysts	Fed 800 fluke cysts	Fed 800 fluke cysts	Fed 800 fluke cysts	Fed 800 fluke cysts
Mar. 15	2	Negative*	Negative	Negative	Negative	Negative
May 7	3	Positive*	Positive	Positive	Positive	Positive
Aug. 3	8	Positive*	Positive	Positive	Positive	Positive
Feb. 18, 1938	13	Positive*	Positive	Positive	Positive	Positive
May 25	16	Negative	Positive	Positive	Positive	Positive
		Considerable reduction of fluke eggs				
June 13	17	Slaughtered. Extensive lesions in liver; 3 immature flukes encapsulated in small bile ducts				
Dec. 14	23		Negative	Negative	Positive	Positive
Jan. 9, 1939	24		Slaughtered. Few lesions in liver. No flukes found			
Jan. 23	24			Slaughtered. Few lesions in liver. No flukes found		
Apr. 17	27				Positive	Positive
Aug. 4	31				Positive	Positive
Jan. 27, 1940	36				Positive	Positive. Very few eggs in feces
Feb. 5	37				Slaughtered. Several lesions in liver; 3 adult flukes found	
May 27	40					Slaughtered. Several lesions in liver; 6 adult flukes found

*Positive or negative for liver-fluke eggs in feces.

Semen Studies in the Bull*

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WITH INCREASING INTEREST being shown in artificial insemination and with the apparent increase in breeding difficulties in dairy cattle, there appears to be much need for a more thorough knowledge of semen.

Studies were started in April 1938 and are still in progress. The results of this work are submitted to supplement similar work[1-7] and to indicate the need for further investigations to determine better methods of evaluating semen quality, especially of bulls.

Other results of semen studies will be included in a report of a coöperative project conducted at Oregon State College.

MATERIAL AND METHODS

These studies included 645 semen samples from 103 bulls. The samples were taken from herd sires throughout western Oregon, from experimental animals and in a few cases from abattoir material.

Semen samples were obtained in the following manner: six from the vagina of cows immediately after service, 158 from the penes of bulls after dismounting after service, two by rectal massage, one from the ampuli of an abattoir specimen. The genital organs of quite a number of bulls were submitted to various laboratory tests, but only one specimen was included in the routine semen-examination work. The remaining 478 samples were taken with an artificial vagina.[8]

The routine laboratory examination of semen included: (1) quantitative estimation of the amount of the ejaculate, (2) microscopic estimation of motility either at the time the sample was taken or within 20 minutes afterward, (3) hydrogen-ion determination, (4) spermatozoa counts tabulated in number per cubic millimeter and (5) tabulation of the number and kinds of abnormal spermatozoa as shown on stained smears.

Throughout the work efforts were made to correlate laboratory findings with actual breeding performances. Because so many factors other than the bull's fertility influence breeding performance in a herd, it is difficult to estimate the extent of the bull's contribution to the situation except in extreme cases.

Work was started in the spring of 1938 and continued for approximately one year. College dairy animals only were used in this work. The seven herd sires and 75 cows used were rather uniformly represented in three different breeds.

Semen samples were taken with an artificial vagina; 1 to 2 cc. of semen was used to impregnate the cows and the remaining portion was taken to the laboratory for routine examination.

All samples were handled alike. At first the artificial vagina was lubricated with vaseline, but this was eventually abandoned, rubber liners being used that had been thoroughly disinfected and dried, without any lubricant. The samples were taken in calibrated centrifuge tubes, protected by a piece of rubber tubing. After the amount was noted, the semen was emptied into sterile 10-cc. test tubes, corked and placed in a box to be taken to the laboratory. It was felt that the mild, even climate made further handling precautions unnecessary.

Motility estimations were recorded as excellent, good, fair, poor or none. Sperm counts were made with hemacytometers and pipettes used in making erythrocyte counts, with 3 per cent NaCl as the diluting fluid. An ordinary blood-smear method was employed in making semen smears, which were stained with a modified eosin-hematoxylin method[9] after being fixed with Schaudinn's solution. The kinds of abnormal forms tabulated included sperm with crooked tails, immature sperm with cytoplasmic drops on the anterior or posterior parts of the middle piece, piriform heads, degenerate forms or sperm showing staining characteristics interpreted as indicating degeneration.

*Technical paper No. 365, Oregon State Agricultural Experiment Station; contribution from the Department of Veterinary Medicine and the Department of Dairy Husbandry.

[1]Donham, C. R., Simms, B. T., and Shaw, J. N.: J. A.V.M.A., lxxviii (May 1931), p. 658.

[2]Moench, G. L.: Am. J. Obst. & Gynecol., xxxii (1936), p. 406.

[3]Nordby, J. E., and Bollen, W. B.: Cornell Vet., xx (1930), p. 381.

[4]Williams, W. W.: Cornell Vet., x (1920), p. 87; xii (1922), p. 19.

[5]Williams, W. W., and Savage, A.: Cornell Vet., xv (1925), p. 253.

[6]Anderson, J.: Vet. J., xcvi (1940).

[7]Miller, F. W., and Evans, E. J.: J. Agr. Res., xlviii (1934), p. 941.

[8]Walton, A.: Notes on Artificial Insemination (Holborn Surgical Instrument Co., Ltd., London, 1936).

[9]Gradwohl, R. B. H.: Clin. Lab. Meth. & Diag. (2nd ed., C. V. Mosby Co., St. Louis, 1938), p. 710.

TABLE I—Results of Semen Evaluation-Artificial Insemination Work Including One Bull and 24 Cows (Bull 1)

DATE	MOTILITY	ABNORMAL (%)	NO. SPERM PER CMM.	COW NO.	PREGNANT	SEMEN PLACEMENT
5- 8-38	Good	10.0	—	292	No	—
5- 3-38	Good	5.8	—	—	No service	—
5- 6-38	Good	10.0	—	327	Yes	—
5-15-38	Good	12.5	—	292	Yes	—
5-27-38	Excellent	10.3	—	340	Yes	—
6- 6-38	Excellent	1.7	—	308	Butchered	—
6-16-38	Excellent	15.0	—	—	No service	—
6-23-38	Poor	16.2	—	342	Yes	—
6-27-38	Poor	24.9	—	317	No	—
7-15-38	None	7.7	—	348	No	—
7-16-38	Poor	10.0	—	343	Yes	—
7-19-38	Fair	29.4	—	339	Yes	—
7-28-38	Good	19.6	—	370	Yes	—
8-18-38	Fair	10.0	—	338	Yes	—
8-24-38	Fair	14.0	—	348	Yes	—
8-29-38	—	9.7	—	323	No	—
1-25-39	Good	8.0	—	355	No	—
2- 8-39	Excellent	—	—	372	Yes	—
2-21-39	Excellent	—	—	358	Yes	—
2-22-39	Excellent	—	—	322	Yes	—
3- 1-39	Good	—	—	347	Yes	—
3- 4-39	Good	—	—	355	Yes	—
4-11-39	Excellent	25.0	—	306	Yes	—
5- 9-39	Good	38.0	—	374	Yes	External os
6- 9-39	Excellent	8.0	—	332	No	Uterus
6-15-39	Excellent	11.0	—	331	Yes	Uterus
7-26-39	Good	—	890,000	—	No service	—
7-28-39	Excellent	—	1,020,000	368	Yes	External os
7-31-39	Excellent	—	1,020,000	376	No	External os
9-25-39	Excellent	6.0	1,420,000	326	Yes	External os

RESULTS

Table I shows the results obtained from one bull and 24 cows inseminated artificially with his semen. No correlation could be noted between semen quality as measured by the laboratory methods used in this study and the percentages of conceptions. The number of individuals, however, was quite small.

Apparently, as shown in table II, there is at times little correlation between the number of abnormal forms and the fertilizing ability of the bull. The principal type of abnormality in this bull's semen was the one designated as crooked tails. An abrupt curve between the middle piece and the tail caused the tails to protrude over the head, causing the sperm to move opposite to the normal direction. This animal also exhibited abnormally low sexual desire.

An evaluation was placed on each sample of semen, rating it as excellent, good, fair or poor. Table III summarizes the results obtained from seven bulls and 75 cows.

Four cows were sold as non-breeders. Table IV summarizes the breeding history of the remaining 71 cows.

One cow conceived from a semen sample that, when examined in the laboratory within 20 minutes after it was taken, showed no motility. As far as was physically possible, all samples were handled alike.

Two bulls were brought to the state college, one as sterile and the other having a poor breeding record. Semen from these two bulls was consistently rated as being poor. Sperm counts were low, motility was very poor, the semen was usually alkaline and upon standing became more alkaline without the usual increase in hydrogen-ion concentration, and the percentages of abnormal spermatozoa were usually quite high though highly variable.

The bull that was considered sterile was

TABLE II—Results of Semen Evaluation-Artificial Insemination Work of the Herd Sire Showing the Poorest Semen (Bull 2)

DATE	MOTILITY	ABNORMAL (%)	NO. SPERM PER CMM.	COW NO.	PREGNANT	SEMEN PLACEMENT
5- 4-38	Poor	83.0	—	—	No service	—
5- 6-38	Poor	52.0	—	128	Yes	—
5-11-38	None	79.0	—	—	No service	—
5-17-38	Fair	91.2	—	130	Yes	—
6- 1-38	Very poor	80.8	—	147	Yes	—
6-25-38	—	27.0	—	126	Yes	—
7-12-38	None	35.8	—	155	No	—
8-30-38	None	80.6	—	148	No	—
8- 8-38	Fair	82.4	—	148	No	—
12- 6-38	Fair	90.0	—	157	No	—
12-28-38	Fair	90.0	—	157	Yes	—
2-28-39	Fair	—	—	150	Yes	—
3-31-39	Poor	—	—	134	No	—
4-15-39	Fair	90.0	—	147	No	Cervix
4-21-39	—	—	—	134	Yes	—
5-20-39	Good	90.0	—	147	No	—
5-21-39	Fair	—	—	141	No	—
7-25-39	Good	—	1,600,000	147	No	External os

in poor condition when brought to the farm. Good feed produced marked physical improvement, but the semen showed little change.

SPECIAL SEMEN STUDIES

Cyclic variations in semen have been observed in small animals by Hammond[10] and in rams by Gunn.[11] Variations were noted in samples taken from the herd sires. To get a more accurate record of such variations and to determine whether or not excessive sexual use was a contributing factor, frequent samples were taken from four bulls: four times daily from two bulls and eight times daily from the other two. The results from two of the bulls are summarized in figures 1 and 2. Apparently, there can be considerable diurnal and daily variation in the characteristics of semen.

After a short period of sexual rest, daily samples were taken from bull 5 for 31 days. As was noted in the previous work, there was considerable variation in the samples. This is shown in figure 3. The bulls used in the above work were all eligible for registration, represented in two breeds, and were all vigorous animals, approximately 2 years of age.

Initial hydrogen-ion concentration and the rapidity with which it changes has been suggested[12] as a possible criterion for evaluating the fertilizing ability of semen.

All hydrogen-ion determinations recorded in this work were made with a potentiom-

[10]Hammond, J.: J. Agr. Sci., xi (1921), pp. 336-337.

[11]Gunn, R. M.: Fertility in sheep. Bul. 94, Counc. Ind. & Sci. Res., Australia (1936), p. 46.

TABLE III—Summary of Semen Evaluation-Artificial Insemination Work Including Seven Bulls and 75 Cows

RATING	NO. OF SAMPLES	NO. PREGNANT	NO. NOT PREGNANT
Excellent	19	10 (52.6%)	9*
Good	27	11 (40.7%)	16†
Fair	21	10 (47.1%)	11‡
Poor	14	7 (50.0%)	7

*Five not pregnant were difficult breeding cows.
†Five not pregnant were difficult breeding cows.
‡Four not pregnant were difficult breeding cows.

[12]Webster, W. M.: Ann. Rpt. Dept. Sci. & Ind. Res., New Zealand, 1938-1939, p. 6.

TABLE IV—Results Summarized in Table III After Deducting Four Cows Sold as Non-Breeders

RATING	NO. OF SAMPLES	NO. PREGNANT	NO. NOT PREGNANT
Excellent	16	10 (62.5%)	6
Good	22	10 (45.4%)	12
Fair	17	11 (64.7%)	6
Poor	12	6 (50.0%)	6

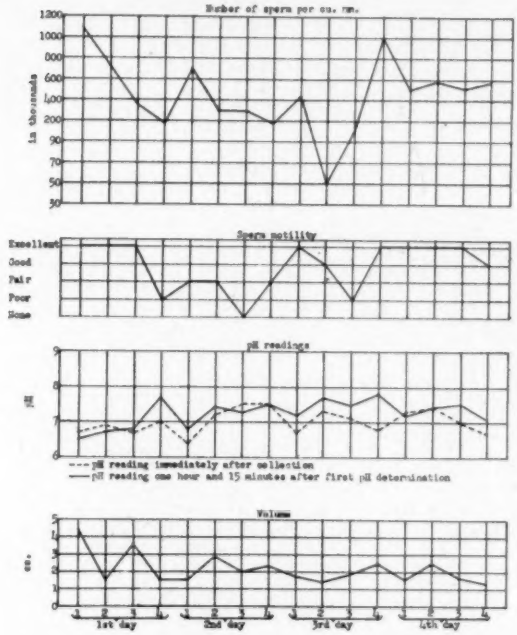

Fig. 1. Semen samples from bull 3. Samples were taken four times daily on April 16-19.

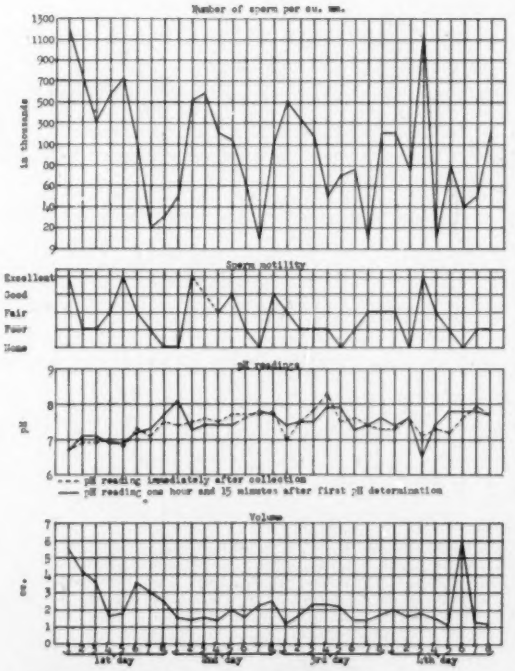

Fig. 2. Semen samples from bull 4. Samples were taken eight times daily on April 16-19.

eter, the glass and calomel electrodes being used.

Figure 4 shows pH comparisons of semen from three bulls.

Increase in hydrogen-ion concentration was more gradual in the sample showing the least motility. Similar results were obtained from many other samples.

Motility, apparently, is one of the factors that cause pH changes in semen. When sperm are inactivated chemically or by refrigeration, the pH curves are very different. This is shown graphically in figures 5 and 6.

Semen from bull 6, considered to be sterile, was compared with semen from bulls 5 and 7. Hydrogen-ion concentrations are compared in figure 7.

Apparently, sperm motility is a factor in causing the hydrogen-ion changes of semen. This is further substantiated by the results shown in figure 6. One sample was divided into equal portions immediately after it was taken, one half being left at room temperature and the other half placed in the refrigerator at 10 C. The refrigerator sample increased in pH. The room-temperature sample showed an initial rise followed by a slow decline. Somewhat similar results were obtained from a like treatment of another sample, one half of which was inactivated with 0.2 cc. of a 1:1,000 solution of bichloride of mercury, the remaining half being left in an open container at room temperature.

pH Determinations of Some of the Components of Semen

The pH determinations shown in table V were made on contents of the ampuli and secretions of the seminal vesicles of bulls. The genital organs were removed immediately after the animals were killed. Some of the readings were made at the abattoir within ten minutes after slaughter and some were made in the laboratory less than 60 minutes afterward.

Insufficient amounts of secretions from the prostate and bulbo-urethral glands made it impossible to make similar deter-

minations. Gross pathology was not noted in any of the organs examined.

ASCORBIC ACID CONTENT OF SEMEN

Phillips[13] has reported that under a variety of conditions a subacute ascorbic acid deficiency may exist. It has been stated that ascorbic acid is intimately involved in the production of viable sperm and closely associated with the physiology of reproduction in the bull.

Some preliminary work has been started to find whether or not there is any correlation between the ascorbic acid content of semen and the breeding efficiency of the bull.

Table VI summarizes results obtained from 17 semen samples from five bulls.*

DISCUSSION

The data that have been presented indicate that semen may be extremely variable in the characteristics that have received attention in this work.

There was a high degree of variation in the amount of the ejaculate. In general, there was a decrease in the quantity of ejaculate when young bulls were allowed to ejaculate several times a day for several successive days. Occasionally after periods of sexual rest the amount of the ejaculate would be quite large, although this was not constant.

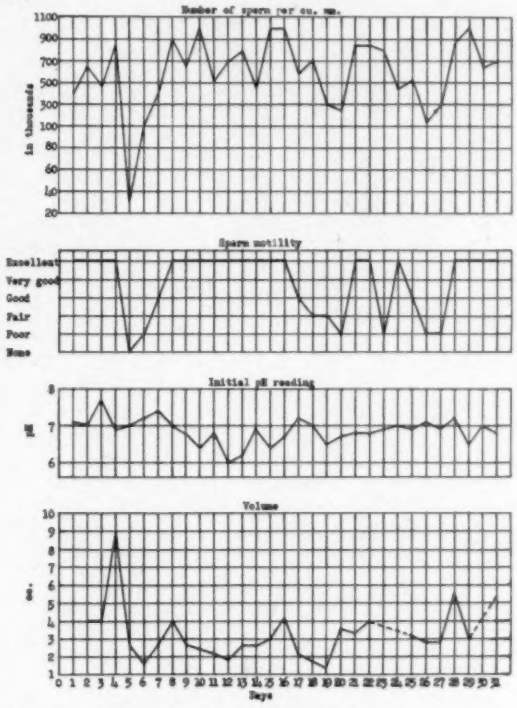

Fig. 3. Semen samples from bull 5. Samples were taken daily for a period of 31 days.

*The ascorbic acid determinations were made by Margaret L. Fincke and Virginia Landquist, Department of Foods and Nutrition, Oregon State College.

[13]Phillips, P. H.: J. Dairy Sci., xxiii (1940), p. 530.

TABLE V—Hydrogen-Ion Concentration of Semen Components

BULL NO.	SEMINAL VESICLE SECRETIONS	AMPULI CONTENTS	AMPULI CONTENTS AND SEMINAL FLUID	WHEN EXAMINED
1	6.01		—	Immediately after slaughter
2	5.96 Pooled	Pooled	—	Immediately after slaughter
3	5.93 sample	sample	—	Immediately after slaughter
4	5.65 5.91	5.92	—	Immediately after slaughter
5	6.01		—	Immediately after slaughter
6	6.39	—	6.47	Less than 60 minutes after slaughter
7	6.18	6.33	6.31	Less than 60 minutes after slaughter
8	6.24	6.26	6.29	Immediately after slaughter
9	5.88	5.66	—	Less than 60 minutes after slaughter
10	5.59	5.97	—	Less than 60 minutes after slaughter
11	6.00	—	—	Less than 60 minutes after slaughter
12	6.01	—	—	Less than 60 minutes after slaughter
13	6.10	6.06	6.17	Immediately after slaughter
14	6.32	6.30	—	Immediately after slaughter
15	6.22	—	—	Immediately after slaughter
16	6.48	—	—	Immediately after slaughter
17	6.10	—	—	Immediately after slaughter

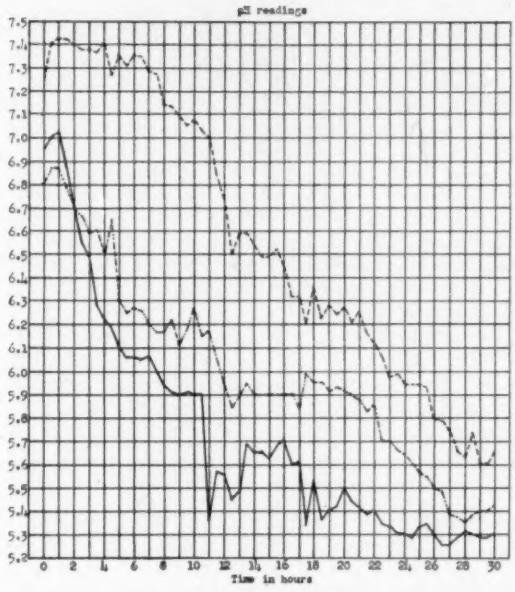

Fig. 4. Immediately after collection the samples were taken to the laboratory, and pH determinations were made at one-half hour intervals for a period of 30 consecutive hours.

- – – Semen from bull 8 taken 4-30-40 at 8:30 a. m. Count: 920,000 sperm per cmm. Fair motility.
- —— Semen from bull 5 taken 4-30-40 at 8:10 a. m. Count: 1,300,000 sperm per cmm. Excellent motility.
- —.. Semen from bull 9 taken 4-30-40 at 8:20 a. m. Count: 1,160,000 sperm per cmm. Excellent motility.

There was considerable variation in the motility of spermatozoa obtained from experimental bulls under heavy sexual use. Semen samples obtained from herd sires used in a regular breeding program showed variations, although the fluctuations were less pronounced and seemingly more seasonal in occurrence. Careful analysis of this work fails to show much correlation between the degree of motility and the percentage of conception; the bull with the most motile sperm, however, was apparently the most fertile. These results should not be accepted too literally, since Spielman,[14] in his analysis of the same herd, showed that breeds and families vary considerably in fertility.

Some interesting things were noted during the routine microscopic examination of semen for motility. First, semen has a tendency to become flocculent when allowed to remain unshaken in test tubes, or in uncovered drops on slides. Second, spermatozoa tend to move in waves or currents. When a series of drops from the same sample were placed on a slide, the currents usually moved in the same direction, commonly counter-clockwise. These characteristics can usually be observed with the

[14]Spielman, A. A., and Jones, I. R.: J. Dairy Sci., xxii (1939), pp. 329-334.

TABLE VI—Ascorbic Acid Content of Semen

Semen Sample No.	Mg. Ascorbic Acid/100 cc.	No. Sperm per cmm.	Motility of Sperm	Breeding Performance
Bull 6				
1	0.97	60,000	None	Poor
2	2.35	110,000	Poor	
3	1.25	125,000	Poor	
4	0.22	110,000	None	
Bull 1				
1	3.33	820,000	Excellent	Very good
2	4.15	1,360,000	Excellent	
3	2.15	330,000	Good	
4	4.52	1,550,000	Good	
Bull 7				
1	5.60	1,240,000	Excellent	Unknown
2	8.15	510,000	Excellent	
3	7.95	420,000	Excellent	
4	6.41	620,000	Excellent	
Bull 10				
1	8.08	370,000	Fair	Unknown
2	5.92	320,000	Excellent	
3	5.47	380,000	Excellent	
Bull G2				
1	4.47	540,000	Fair	Poor
2	5.71	480,000	Fair	

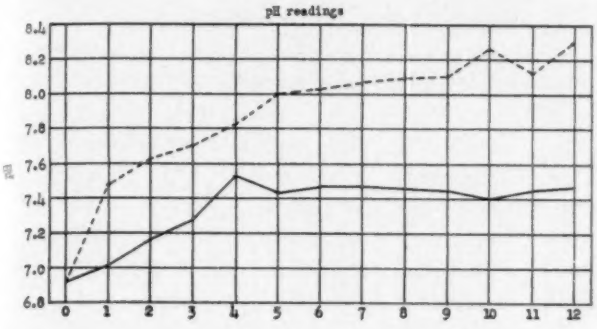

Fig. 5. Semen from bull 5 taken 5-3-40. Count: 535,000 sperm per cmm. Excellent motility. Immediately after ejaculation a pH determination was made. Then, one half of the sample was held at room temperature (27-29 C.) and the other half placed in a refrigerator and held at 10 C. pH determinations were made every half hour for a period of six hours.

--- Refrigerator.
—— Room temperature.

naked eye and were most prominent in samples having a high degree of motility and with high sperm counts. Similar characteristics were noted in the contents of some of the ampuli. Action in these cases was more violent, but did not persist as long, when placed in drops on slides at room temperature. These phenomena need careful study, and may be of considerable physiologic importance.

Hydrogen-ion variations were quite prominent, but very difficult to interpret. Experience indicates that pH determinations of semen samples should be made as soon as possible after taking. Relatively rapid changes in pH occur in samples exposed to air at ordinary room temperatures. When very motile samples with high sperm counts were placed in open containers at room temperature, and pH readings were made over a period of several hours, there was an increase in hydrogen-ion concentration. Samples showing poor motility and samples from two animals known to be of lowered fertility did not show this steady decline in pH. Some of them became more alkaline and remained at the higher pH levels. If pH values are included in semen examinations, the first reading should be made immediately after the sample is taken and then a series of recordings should be made over a period of six to twelve hours. Such records should be of definite value.

Spermatozoa counts were variable. Less variation was noted in the herd sires on a regular breeding program than in young bulls in heavy sexual use. In samples from the latter animals there was some correlation between sperm counts and motility. When the counts were very low, motility

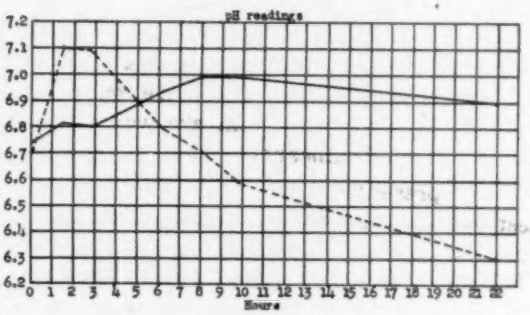

Fig. 6

--- Check 7.
—— Sperm from bull 7 were inactivated with 0.2 cc. of bichloride of mercury (1:1,000).

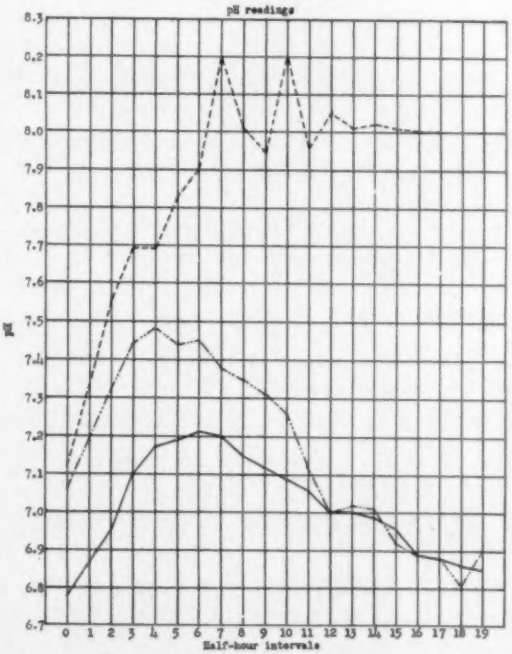

Fig. 7. pH readings were made on the semen immediately after collection and at half-hour intervals thereafter.

--- Bull 6; proved to be sterile.
-.-.- Bull 5.
—— Bull 7.

was usually poor. An even distribution is important in making counts. Some difficulty was experienced in getting such distribution because of the tendency for spermatozoa to agglutinate. This is especially true of the very motile semen samples. A more even distribution of sperm can be attained by placing a tube containing the semen in a water bath of 56 C. for at least five minutes. The stem of the diluting pipette will then be filled with a more representative part of the sample.

In this work little significance could be attached to the kinds and percentages of abnormal spermatozoa, counted on stained slides. In the slides examined there were very few of the anomalies that are usually considered most significantly pathologic.

The data presented indicate that no single test can be used in evaluating the fertilizing ability of semen and that, when semen is subjected to all the common laboratory tests, such evaluations are of doubtful value except in extreme cases.

These data further show that daily and diurnal variations in the characteristics of semen render such evaluations as present methods permit practically impossible without making a series of examinations.

It is hoped that this work will stress the shortcomings of our present knowledge of semen and emphasize the need for a more thorough knowledge of semen physiology and pathology.

SUMMARY

1) Over a period of two years 645 semen samples were examined. Of these, 478 were taken by the use of an artificial vagina.

2) Routine laboratory examination of semen included: (1) amount of ejaculate, (2) motility estimations, (3) hydrogen-ion determinations, (4) spermatozoa counts and (5) tabulation of number and kinds of abnormal spermatozoa.

3) Seventy-five cows were inseminated artificially with semen from seven bulls. The semen was collected with an artificial vagina and part of each sample was examined in the laboratory. Little correlation was noted between breeding efficiency and laboratory findings.

4) There is a correlation between hydrogen-ion changes of semen in the first few hours after it has been taken and motility and viability of spermatozoa. Very motile samples usually decrease in pH more rapidly than samples with poor motility.

5) Some factors (refrigeration, chemical inactivation) that inhibit motility will prevent these rapid decreases in pH.

6) Semen samples from two bulls known to have had lowered fertility were usually alkaline and showed a rise, rather than a fall, in pH.

7) Seminal vesicle secretions from abattoir specimens varied in pH from 5.65 to 6.33.

8) When judged by the methods of semen examinations used in this work, there are considerable daily and diurnal variations in semen quality, these being more marked in samples obtained from bulls in heavy sexual use.

9) The ascorbic acid content of semen varied from 0.97 mg. to 8.08 mg. per 100 cc. of semen. Analyses were made of 17 samples obtained from five bulls. The ascorbic acid content of semen obtained from one bull with very poor breeding records was lower than that of semen from bulls with good breeding records. The ascorbic acid content from one bull with a rather poor breeding record was quite high, only two determinations being made.

Connective Tissue Tumors of Horses and Mules*

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AMONG THE TUMORS removed from horses and mules in the Iowa State College clinic and submitted to the pathology department for diagnosis during the past 20 years, 156 originated from the various types of connective tissue: fibrous connective tissue, mucous connective tissue, adipose tissue, bone and cartilage, or combinations of these tissues. Of these 156 neoplasms, 65 were benign and 91 malignant. The benign tumors were, therefore, fibromas, myxomas, lipomas, osteomas or chondromas, or combinations of these five types. The malignant tumors were varieties of sarcomas, the exact designation depending upon the type of parent tissue, *e.g.*, fibrosarcoma and myxosarcoma.

The study of the 156 tumors embraced many of the factors usually included in an investigation of neoplasms, but since the age incidence of the animals involved, the histogenic classification of the tumors, the anatomic location of them and their size are the elements of most general interest, only these four will receive major consideration.

BENIGN CONNECTIVE TISSUE TUMORS

The benign connective tissue tumors were relatively slow growing, more or less encapsulated and, when located on the surface of the body, frequently were not covered with skin. Microscopically their cells were more or less mature. At least, they were not young enough to classify as embryonic and, furthermore, cell-division figures were absent or so rare that their presence attracted no attention.

Age was not a factor in their occurrence. Colts from 4 months of age to horses 26 years old furnished specimens for the study.

Fibromas.—Thirty-two benign, fibrous, connective tissue tumors from 31 horses and mules were located principally on the legs, the head, the pectoral and shoulder regions, and in the nasal cavity. The order in which the locations are listed corresponds to the frequency of tumor occurrence in these parts.

In nine instances the tumors were associated with sites of previous injury: rope burns, collar galls, manger contusions, plow cuts, the spontaneous disappearance and surgical removal of papillomas. This suggests that the connective tissue masses in these places may have been older granulation and young, scar tissue rather than strictly neoplastic tissue.

A differentiation between only slightly infected, moderately mature granulation tissue and a fibroma is at times quite difficult. Likewise an infected fibroma sometimes has the appearance of granulation tissue. Five nasal fibromas were chiefly the soft type (nasal polyps). A 19-month-old colt furnished one of these. The largest fibroma, located at the elbow region, was completely covered with skin and measured approximately 18 cm. in diameter.

Myxofibroma.—A subcutaneous tumor of mucous connective tissue and fibrous connective tissue from a 4-month-old colt and a growth of the same kind from a maxillary sinus of an adult horse of unknown age were the only representatives of this class of neoplasms examined.

Osteofibromas.—Nine tumors composed of a combination of osseous and fibrous connective tissue all had their location somewhere on the head except one. This one, which was about 18 to 20 cm. in diameter, was removed from the right hock. It had a history of having occurred at the site of a wire cut. Two of the osteofibromas developed on the mandible and two on a maxilla. The remaining four were located in the maxillary sinuses. One of these extended over into the frontal sinus.

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Osteomyxofibromas.—The turbinates and the nasal sinuses were the favorite locations for our three specimens of tumors composed of a combination of bone, mucous connective tissue and fibrous connective tissue.

Myxoma.—Several curettements, varying in size up to 12 mm. in diameter, removed from the maxillary sinus of a horse furnished our only tissue which could be diagnosed as originating from pure, mucous connective tissue. The surface of the curettements was smooth. They were soft, lobulated, reddish-yellow, translucent and somewhat gelatinous.

Fibromyxomas.—Two mules and a horse supplied three of these neoplasms. In the mules the location was subcutaneous: in one on the "side" and in the other somewhere on a leg. The one from the side measured 10 x 12 x 14 cm. The one from the horse was removed from a nostril, but dimensions were not recorded.

Osteomyxomas.—The nasal passages were the site of predilection for the three specimens we have of these tumors. One originated in the nasal passage proper, one in the frontal sinus and one in a maxillary sinus. The osteomyxoma from the nasal cavity was attached high up and its anterior end protruded from the nostril. It measured 32 cm. in length and 8 cm. in its greatest diameter.

Osteofibromyxomas.—Four tumors consisting of a combination of bone, fibrous connective tissue and mucous connective tissue had their origin in the nasal cavity. The largest weighed 350 Gm. and measured 13 x 8 x 4 cm.

Lipomas.—A pure lipoma and a fibrolipoma had their location in the abdominal cavity. The lipoma was attached to the mesentery, whereas the fibrolipoma was free in the abdominal cavity and presented no evidence of recent attachment. Its center was necrotic. An osteolipoma had its attachment on one of the turbinated bones. It contained enough fat to cause it to float on water.

Osteoma.—The only pure osteoma presented for diagnosis consisted of a mass of spongy bone, 8 x 8 x 4.5 cm., which had been removed from the right premaxilla of a 4-year-old gelding. The tumor was attached to the bone just under the lip and had been developing for about a year. Microscopically a fringe of prominent osteoblasts around the bone masses indicated that the tumor was actively developing.

Fibroösteoma. — Three fibroösteomas were removed from the jaws: two from the mandible and one from a maxilla. One on the mandible measured 10 x 5 x 2.5 cm.

Fibromyxoösteoma. — The only representative of this class of neoplasms in the collection was one 9 x 7 x 6 cm., taken from the under surface of the tail of an 11-year-old mare. It had its origin on a coccygeal vertebra and protruded through the hairless skin underneath the tail.

Fibrochondroma.—The only specimen of a tumor composed of cartilage submitted to the laboratory was removed surgically from the anterior part of the nasal septum. It extended transversely from the ventral margin of the left nostril to the dorsal margin of the right nostril.

MALIGNANT CONNECTIVE TISSUE TUMORS: SARCOMAS

The malignant connective tissue tumors had a history of more rapid growth than the benign ones, although in several instances the records indicated that the neoplasms had been developing slowly for as long as one or two years when suddenly their growth became greatly accelerated. The alarming rate of growth was usually the factor which induced the owner to have the tumor removed. Microscopically the latter type of tumor usually showed islands of embryonic tissue growing in the midst of more adult tissue of the same connective tissue type, thus indicating a possible transformation of a benign to a malignant tumor. The majority of the sarcomas, however, developed rapidly from the beginning, and practically all of their cells were immature, but their degree of immaturity was variable. Cell-division figures could often be seen, but such figures never were as prominent as they usually are in malignant epithelial tumors (carcinomas).

The 91 sarcomas were removed from 30

animals: 65 horses and 15 mules. Eighty-one of these tumors were fibrosarcomas, one a myxosarcoma, five myxofibrosarcomas, two osteofibrosarcomas, and one a myxofibroösteosarcoma. Over one half of them were obtained from animals 1 to 4 years of age, inclusive. A colt 4 months old was the youngest animal from which a sarcoma was removed and a 15-year-old mare the oldest.

Eleven of the sarcomas were recurrences; one had recurred twice. Dr. M. J. Johnson of the clinic believes our records with regard to recurrences are incomplete—that a larger number of recurrences have appeared than our records show. In only two cases did the report from the clinic state that the regional lymph nodes were enlarged. Even in these cases the enlargement may have been due to lymphadenitis rather than metastasis.

As would be expected, there was no uniformity in the size of the sarcomas. With reference to size, the greatest interest naturally centers on extremely large tumors. Among those examined, the largest weighed 14.5 kg. (31.9 lb.) and measured 65x33x16 cm. It was removed from the region of the throat and involved the lower jaw and upper cervical region of a 4-year-old mule (fig. 1). The owner claimed that the tumor was present at birth. A case in which alarming symptoms arose from the presence of a large tumor was seen in a 2-year-old colt in which a spherical fibrosarcoma about 15 cm. in diameter developed in a maxillary sinus. The tumor had been developing only three months. A similar but slightly smaller tumor of two-month duration appeared on the bridge of the nose of the same colt.

Anatomically the sites for the sarcomas were:

Ear	3
Eye lid	5
Face	10
Lower jaw	6
Mouth	4
Nasal passages and sinuses	5
Nostril	2
Neck, shoulders, prepectoral region	13
Limbs	28
Abdominal wall	11
Tail	1
Specific location not recorded	3

SUMMARY

1) A total of 156 connective tissue tumors removed from horses and mules at the Iowa State College clinic are briefly reported.

2) Benign neoplasms were encountered 65 times and malignant ones 91 times.

3) In no particular age group was the incidence of benign connective tissue tumors significantly higher than in other age groups.

4) More than one half of the sarcomas occurred in horses between 1 and 4 years of age, inclusive.

5) Fibromas constituted more than 50 per cent of the benign tumors.

6) Fibrosarcomas led the incidence rate among the malignant tumors with a percentage of 89.

7) Fibromas often had their origin at sites of previous injury.

8) The head and nasal passages were the seats of most common occurrence for

—Photo by George R. Fowler.

Fig. 1. A 32-lb. fibrosarcoma on the head and neck of a 4-year-old mule.

Studies on Equine Encephalomyelitis. I. Antigenic Properties of Pigeon-Brain-Tissue Vaccine*

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SINCE Shahan and Giltner[1] protected horses against equine encephalomyelitis by the injection of formalized horse-brain-tissue virus, immunization procedures have been markedly improved. The first step in obtaining a highly concentrated encephalomyelitis virus *in vivo* was made by Higbie and Howitt[2] in propagating the virus of equine encephalomyelitis in the developing chick embryo. However, it remained for Beard, Finkelstein, Sealy and Wyckoff[3] to recognize the advantage of the developing chick embryo in the preparation of a highly antigenic equine encephalomyelitis vaccine. In critical laboratory tests as well as in the field the virus in chick embryos following inactivation by the addition of formalin has proved a superior immunizing agent.

In this connection, Mitchell, Walker and Plummer[4] found 5,000 guinea pig lethal doses of virus per gram of horse-brain tissue, 15,000 lethal doses per gram in the brain of guinea pigs, 5 million per gram in the brain of the mouse and 5 billion per gram in the chick embryo. It, therefore, might appear that the horse-brain vaccine possesses irregular or incomplete protective properties due to its low virus content.

One aspect of studies on equine encephalomyelitis virus at the Illinois experiment station has been the virus content of pigeon-brain tissue. Giltner and Shahan[5] reported that pigeons were susceptible to equine encephalomyelitis virus upon intracranial inoculation. Following these observations pigeons often were employed in our laboratory in the routine isolation of encephalomyelitis virus (western) from natural outbreaks of the disease in horses. Pigeons also appear suitable for the isolation of the eastern virus, since Fothergill and Dingle[6] of Massachusetts reported a natural outbreak of encephalomyelitis in pigeons due to the eastern type. In connection with the use of pigeons in the isolation of equine encephalomyelitis virus in Illinois, an opportunity was afforded to note the concentration of western virus in the brain tissue of pigeons which succumbed following intracranial inoculation.

The purpose of this paper is to note the successful passage of a western equine encephalomyelitis virus in pigeons, by intracranial inoculation of 0.1 cc. of a 1:1,000,000 dilution of pigeon-brain-tissue virus. A series of more than ten successive passages of virus in the brain of pigeons suggested multiplication of the virus, since the brain tissue of intracranially inoculated pigeons revealed an equivalent of 5 billion

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[1]Shahan, M. S., and Giltner, L. T.: Some aspects of infection and immunity in equine encephalomyelitis. J.A.V.M.A., lxxxiv (1934), p 928.

[2]Higbie, Elizabeth, and Howitt, Beatrice: The behavior of the virus of equine encephalomyelitis on the chorioallantoic membrane of the developing chick. J. Bact., xxxix (1935), pp. 399-406.

[3]Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G.: Immunization against equine encephalomyelitis with chick embryo vaccines. Sci., lxxxvii (1938), p. 490.

[4]Mitchell, C. A., Walker, R. V. L., and Plummer, P. J. G.: Studies in equine encephalomyelitis. Chick embryo vaccine as a protective agent. Canadian J. Comp. Med., xxi (1938), pp. 1-12.

[5]Giltner, L. T., and Shahan, M. S.: Transmission of infectious equine encephalomyelitis in mammals and birds. Sci., lxxviii (1933), pp. 63-64.

[6]Fothergill, L. D., and Dingle, J. H.: A fatal disease of pigeons caused by the virus of the eastern variety of equine encephalomyelitis. Sci., lxxxviii (1938), pp. 549-550.

(*Continued from preceding page*)

more than one half of the benign tumors.

9) Among the sarcomas 39 per cent were removed from the head and nasal passages, 30 per cent from the limbs and the remaining 31 per cent from scattered regions of the surface of the body.

TABLE I—Titration of Strain 6289 Western Equine Encephalomyelitis Virus from Pigeon Brain (Lot A—Composite of 25 Pigeon Brains—Eleventh Transfer)

Pigeon No.	Dilution	Result: Days After Inoculation									
		1	2	3	4	5	6	7	8	9	10
1	1:1,000,000	H	H	H	S	D	..	..	..	..	..
2	1:1,000,000	H	H	S	D	..	..	..	..	..	..
3	1:1,000,000	H	S	D	..	..	..	..	..	..	..
4	1:10,000,000	H	S	D	..	..	..	..	..	..	..
5	1:10,000,000	H	H	H	H	S	S	S	S	D	..
6	1:50,000,000	H	H	S	D	..	..	..	..	..	..
7	1:50,000,000	H	H	S	D	..	..	..	..	..	..
8	1:50,000,000	H	H	H	S	D	..	..	..	..	..
9	1:100,000,000	H	H	S	D	..	..	..	..	..	..
10	1:100,000,000	H	S	D	..	..	..	..	..	..	..
11	1:200,000,000	H	H	S	D	..	..	..	..	..	..
12	1:500,000,000	H	H	S	D	..	..	..	..	..	..
13	1:500,000,000	H	H	H	H	H	H	H	S	S	D
14	1:1,000,000,000	H	H	H	H	H	H	H	H	H	H
15	1:1,000,000,000	H	H	H	H	H	H	H	H	H	H
16	1:1,000,000,000	H	H	H	H	H	H	H	H	H	H
17	1:1,000,000,000	H	H	H	H	H	H	H	H	H	H

*0.1 cc. inoculated intracerebrally into each pigeon.
H = healthy, S = sick, D = dead.

intracranial pigeon lethal doses of virus per gram of brain tissue. In the brain of the average pigeon (1.75 Gm.) 8,750,000,000 intracranial pigeon lethal doses of tissue virus were found. The results of titration of a composite pigeon-brain-tissue virus showed that (lot A, strain 6289) 0.1 cc. of a dilution of 1:500,000,000 injected intracranially was lethal to pigeons (table I). The minimum lethal dose of pigeon-brain-tissue virus (lot A, strain 6289) in guinea pigs was found to be 0.1 cc. of a dilution of 1:10,000,000 following intracranial inoculation. Stated in another way, pigeon-brain-tissue virus (lot A, strain 6289) contained 100 million guinea pig lethal doses per gram, or approximately 50 times less than reported in chick-embryo tissue, though it was 20,000 times more concentrated than in horse-brain tissue, 6,667 times more concentrated than in guinea pig-brain tissue, and 20 times more concentrated than in mouse-brain tissue when compared with the ranges of the virus concentration reported by Mitchell, Walker and Plummer.[4]

The virus concentration encountered in pigeon-brain tissue following intracranial inoculation suggested an inquiry regarding the antigenic properties of this tissue virus following inactivation. For this purpose a 20 per cent tryptone broth suspension of the pigeon-brain virus (lot A, strain 6289) was inactivated at room temperature for 48 hours by the addition of 0.5 per cent formalin. Following safety and purity tests of the experimental vaccine for the presence of active virus and bacteria, respectively, a series of twelve guinea pigs were injected subcutaneously at weekly intervals with two 0.5-cc. doses of the formalized pigeon-brain vaccine. Two weeks after the second dose of vaccine was administered, the vaccinated guinea pigs, together with four unvaccinated controls were exposed intracranially by inoculating each with 0.1 cc. of a 1:500 suspension of unaltered equine encephalomyelitis pigeon-brain virus. All of the vaccinated guinea pigs survived and all of the controls succumbed (table II).

To ascertain further the protective properties of formalized pigeon-brain vaccine (lot A, strain 6289), a series of six pigeons were given two successive doses of 0.5 cc. subcutaneously at weekly intervals,

TABLE II—Test of Immunizing Properties of Strain 6289 Western Equine Encephalomyelitis Pigeon-Brain Vaccine

Guinea Pig No.	Vaccine Lot A	Dosage		Virus Exposure	Result									
					Days After Inoculation									
		1st	2nd		1	2	3	4	5	6	7	8	9	10
1	20% formalized pigeon-brain vaccine (lot A, composite of 25 pigeon brains—11th transfer)	0.5 cc. subcutaneously to each guinea pig	7 days later 0.5 cc. subcutaneously to each guinea pig	Intracranial inoculation 0.1 cc. pigeon-brain virus (strain 6289), dilution 1:500, 14 days after second dose of vaccine	H	H	H	H	H	H	H	H	H	H
2					H	H	H	H	H	H	H	H	H	H
3					H	H	H	H	H	H	H	H	H	H
4					H	H	H	H	H	H	H	H	H	H
5					H	H	H	H	H	H	H	H	H	H
6					H	H	H	H	H	H	H	H	H	H
7					H	H	H	H	H	H	H	H	H	H
8					H	H	H	H	H	H	H	H	H	H
9					H	H	H	H	H	H	H	H	H	H
10					H	H	H	H	H	H	H	H	H	H
11					H	H	H	H	H	H	H	H	H	H
12					H	H	H	H	H	H	H	H	H	H
13	Controls; no vaccine				H	H	H	S	S	S	D	.	.	.
14					H	H	H	S	S	S	D	.	.	.
15					H	H	H	S	D	.	.	.	.	.
16					H	H	H	S	S	D	.	.	.	.

H = healthy, S = sick, D = dead.

while another series of six pigeons received two 1-cc. doses. Fourteen days later the vaccinated pigeons together with four unvaccinated controls received 0.1 cc. of a 1:500 dilution of pigeon-brain-tissue virus (6289) intracranially. All six of the pigeons receiving the 0.5-cc. doses succumbed within ten days following intracranial exposure, while only two of the six pigeons receiving two 1-cc. doses of vaccine succumbed. All of the controls died on the third day following exposure (table III).

The same lot of experimental pigeon-brain vaccine was held at ice-box tempera-

TABLE III—Test of Immunizing Properties of Strain 6289 Western Equine Encephalomyelitis Pigeon-Brain Vaccine

Pigeon No.	Vaccine Lot A	Dosage		Virus Exposure	Result									
					Days After Exposure									
		1st	2nd		1	2	3	4	5	6	7	8	9	10
1	20% formalized pigeon-brain vaccine (lot A, composite of 25 pigeon brains—11th transfer)	0.5 cc. subcutaneously to each pigeon	7 days later 0.5 cc. subcutaneously to each pigeon	Intracranial inoculation 0.1 cc. pigeon-brain virus (strain 6289), dilution 1:500, 14 days after second dose of vaccine	H	H	H	H	H	H	S	D	.	.
2					H	S	D	.	.	.	.	.	.	.
3					H	H	S	D	.	.	.	.	.	.
4					H	H	H	S	D	.	.	.	.	.
5					H	H	H	H	H	H	H	H	H	H
6					H	H	H	H	S	D	.	.	.	.
7		1 cc. subcutaneously to each pigeon	7 days later 1 cc. subcutaneously to each pigeon		H	H	S	D	.	.	.	.	.	.
8					H	S	D	.	.	.	.	.	.	.
9					H	H	H	H	H	H	H	H	H	H
10					H	H	H	H	H	H	H	H	H	H
11					H	H	H	H	H	H	H	H	H	H
12					H	H	H	H	H	H	H	H	H	H
13	Controls; no vaccine				H	S	D	.	.	.	.	.	.	.
14					H	S	D	.	.	.	.	.	.	.
15					H	S	D	.	.	.	.	.	.	.
16					H	S	D	.	.	.	.	.	.	.

H = healthy, S = sick, D = dead.

ture (approximately 45 F.) and subjected to two additional guinea pig-potency tests at intervals of 30 days. In the first of the two retests eleven guinea pigs weighing 500 Gm. or more were employed, since smaller guinea pigs (400 Gm.), as employed in the initial test, were not available. The protective properties of the vaccine in the heavier guinea pigs were noted following two 0.5-cc. doses of the vaccine administered subcutaneously at weekly intervals, followed by a challenging dose of 0.1 cc. of a 1:500 dilution of pigeon-brain virus administered intracranially. One of the eleven vaccinated guinea pigs succumbed to trauma, while three of the remaining ten guinea pigs succumbed to the challenging dose of virus. All four controls died on or before the seventh day. Since seven of the vaccinated guinea pigs survived, it is apparent that a measurable degree of protection was provided in guinea pigs which weighed 500 Gm. or more at the beginning of the test (table IV).

One month later a third potency test on twelve 400-Gm. guinea pigs was conducted in the manner described above. One pig died of trauma, while four of the eleven vaccinated animals succumbed. All four of the controls succumbed on or before the ninth day following exposure (table V).

Further experimental evidence on the protective or antigenic character of formalized pigeon-brain virus (strain 6289) was obtained with a separate lot of vaccine, designated lot B, strain 6289. This experimental vaccine was prepared from the brains of six moribund pigeons suffering from encephalomyelitis following intracerebral inoculation with 0.1 cc. of a dilution of 1:1,000,000 of pigeon-brain-tissue virus. A 20 per cent brain-tissue vaccine (lot B, strain 6289) was inactivated in the same way as lot A, strain 6289. Twelve guinea pigs (400 Gm.) were vaccinated and exposed as in preceding potency tests. Two vaccinated guinea pigs were apparently not protected. The remaining seven vaccinated guinea pigs survived, while all four controls succumbed (table VI).

Further observations on the antigenic properties of formalized pigeon-brain-tissue virus were made on a third experimental lot of vaccine, designated lot C, strain 6289. This vaccine was prepared from a pool of 20 pigeon brains by the method employed for lots A and B. Before inactivation the virus content was found on titration to be approximately 100 mil-

TABLE IV—Test of Immunizing Properties of Strain 6289 Western Equine Encephalomyelitis Pigeon-Brain Vaccine

Guinea Pig No.	Vaccine Lot A	Dosage		Virus Exposure	Result: Days After Exposure									
		1st	2nd		1	2	3	4	5	6	7	8	9	10
1	20% formalized pigeon-brain vaccine (lot A, composite of 25 pigeon brains—11th transfer)	0.5 cc. subcutaneously to each guinea pig	7 days later 0.5 cc. subcutaneously to each guinea pig	Intracranial inoculation 0.1 cc. pigeon-brain virus (strain 6289), dilution 1:500, 14 days after second dose of vaccine	H	H	H	H	H	S	S	D	. .	. .
2					H	H	H	H	H	H	H	H	H	H
3					H	H	H	H	H	H	H	H	H	H
4					H	H	H	H	H	H	H	H	H	H
5					H	H	H	H	H	S	D	. .	. .	. .
6					H	H	H	H	H	S	S	D	. .	. .
7					T	. .	. .	. .	. .	. .	. .	. .	. .	. .
8					H	H	H	H	H	H	H	H	H	H
9					H	H	H	H	H	H	H	H	H	H
10					H	H	H	H	H	H	H	H	H	H
11					H	H	H	H	H	H	H	H	H	H
12	Controls; no vaccine				H	H	S	S	S	S	D	. .	. .	. .
13					H	H	S	S	S	S	D	. .	. .	. .
14					H	H	S	S	S	D	. .	. .	. .	. .
15					H	H	S	S	S	S	D	. .	. .	. .

H = healthy, S = sick, D = dead, T = trauma.

TABLE V—Test of Immunizing Properties of Strain 6289 Western Equine Encephalomyelitis Pigeon-Brain Vaccine

Guinea Pig No.	Vaccine Lot A	Dosage		Virus Exposure	Result									
					Days After Exposure									
		1st	2nd		1	2	3	4	5	6	7	8	9	10
1	20% formalized pigeon-brain vaccine (lot A, composite of 25 pigeon brains—11th transfer)	0.5 cc. subcutaneously to each guinea pig	7 days later 0.5 cc. subcutaneously to each guinea pig	Intracranial inoculation 0.1 cc. pigeon-brain virus (strain 6289), dilution 1:500, 14 days after second dose of vaccine	H	H	H	H	H	H	H	H	H	H
2					H	H	H	H	H	H	H	H	H	H
3					H	H	H	H	H	H	H	H	H	H
4					H	H	H	H	H	H	H	H	H	H
5					H	H	H	H	H	H	H	H	H	H
6					H	H	H	H	H	H	H	H	H	H
7					H	H	H	H	H	H	H	S	H	H
8					H	H	H	H	S	S	S	D	.	.
9					H	H	H	H	H	H	S	D	.	.
10					H	H	H	D	.	.	.	.	.	.
11					H	H	H	S	S	D	.	.	.	.
12					T	T	.	.	.	.	.	.	.	.
13	Controls; no vaccine				H	H	H	H	S	S	S	D	.	.
14					H	H	H	S	S	S	S	D	.	.
15					H	H	H	H	S	D	.	.	.	.
16					H	H	H	H	S	S	S	S	D	.

H = healthy, S = sick, D = dead, T = trauma.

lion guinea pig lethal doses and 5 billion to 10 billion pigeon lethal doses per gram. Three separate guinea pig-potency tests were conducted with twelve vaccinated guinea pigs in each as in the potency tests of lots A and B. In the first test three vaccinated guinea pigs died following the administration of the challenging dose of virus. In the second test one animal died of trauma and three other vaccinated guinea pigs died following exposure. In the third test two guinea pigs died of trauma,

TABLE VI—Test of Immunizing Properties of Strain 6289 Western Equine Encephalomyelitis Pigeon-Brain Vaccine

Guinea Pig No.	Vaccine Lot B	Dosage		Virus Exposure	Result												
					Days After Exposure												
		1st	2nd		1	2	3	4	5	6	7	8	9	10	11	12	13
1	20% formalized pigeon-brain vaccine strain 6289 (composite of 6 pigeon brains)	0.5 cc. subcutaneously to each guinea pig	7 days later 0.5 cc. subcutaneously to each guinea pig	Intracranial inoculation 0.1 cc. pigeon-brain virus (strain 6289), dilution 1:500, 14 days after second dose of vaccine	T	.	.	.	.	.	.	.	.	.	.	.	.
2					H	H	H	H	H	H	H	H	H	H	H	H	H
3					T	.	.	.	.	.	.	.	.	.	.	.	.
4					H	H	H	H	H	H	H	H	H	H	H	H	H
5					H	H	H	H	H	H	H	H	H	H	H	H	H
6					H	H	H	H	H	S	S	S	S	S	S	S	D
7					H	H	H	H	H	S	S	S	S	S	S	D	.
8					H	H	H	H	H	H	H	H	H	H	H	H	H
9					H	H	H	H	H	S	H	H	H	H	H	H	H
10					H	H	H	H	H	S	H	H	H	H	H	H	H
11					T	T	D	.	.	.	.	.	.	.	.	.	.
12					H	H	H	H	H	H	H	H	H	H	H	H	H
13	Controls; no vaccine				H	H	H	H	H	S	S	S	S	S	S	D	.
14					H	H	S	S	S	S	D	.	.	.	.	.	.
15					H	H	H	H	H	D	.	.	.	.	.	.	.
16					H	H	H	H	H	S	D	.	.	.	.	.	.

T = trauma, H = healthy, S = sick, D = dead.

but the remaining ten vaccinated animals were released healthy two weeks after intracranial exposure. The control animals in each of the three tests all died following exposure. These results of potency tests on experimental vaccine lot C, strain 6289, are in complete accord with those obtained with experimental vaccines lots A and B of the same strain of virus.

SUMMARY

Equine encephalomyelitis pigeon-brain virus (western strain 6289) was passaged through ten consecutive pigeon transfers by intracranial inoculation of 0.1 cc. of a dilution of 1:1,000,000. A titration of the pigeon-brain-tissue virus intracranially revealed approximately 5 billion pigeon lethal doses and 100 million guinea pig lethal doses of virus per gram.

Three separate lots of pigeon-brain-tissue virus inactivated by the addition of 0.5 per cent formalin showed a measurable degree of immunizing power in guinea pigs and also in pigeons following subcutaneous administration.

A New Service of "Biological Abstracts"

To meet the requirements of men engaged in the animal industries, *Biological Abstracts* announces the establishment of a new section, Section F, Abstracts of Animal Production and Veterinary Science, beginning January 1942.

The biologic research literature on the breeding, nutrition, husbandry, diseases and pests of the domesticated animals—including poultry, fur-bearing animals and pet stock—is scattered throughout a large number of original research journals in many languages, and its assembly in the abstract issues and indexes of a single comprehensive abstracting journal will, it is felt, be a great convenience for all workers in animal production.

The new section will consist of ten abstract issues per year. The annual subscription rate will be $5. Subscribers will receive the index to the complete edition of *Biological Abstracts*.

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Inquiries should be addressed to *Biological Abstracts*, University of Pennsylvania, Philadelphia, Pa.

Pregnancy Diagnosis in Swine by a Chemical Test*

S. Y. ROTH, B.S., D. T. MAYER, A.B., A.M., Ph.D., and RALPH BOGART, B.S., M.S., Ph.D.
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PREGNANCY TESTS IN farm animals have become increasingly important to the livestock breeder and research worker. Acceptable tests have been developed for detecting pregnancy in the genus Equus (horse, zebra and ass), but no satisfactory test has yet been made available for diagnosing pregnancy in swine. The purpose of this study was to develop a practical and accurate test for diagnosing pregnancy in swine early in the gestation period which might be useful in experimental studies such as are in progress in the Regional Swine Breeding Laboratory.

Faiermark and Bigos,[1] Faiermark[2] and Kust and Struck,[3] using the biologic method of assay on the urine of swine, detected estrogens from the third to the fifth week and from the eleventh week of gestation to term. These workers found no greater quantity of estrogens in the urine of pregnant sows from the fifth to the eleventh week of gestation than were present in the nonpregnant female.

EXPERIMENTAL PROCEDURE

Since the urine of the sow differs from that of the mare in that it contains more pigments, which interfere with the reading of the Cuboni test,[4] certain modifications were necessary in order to adapt the test for pregnancy diagnosis in the sow. Efforts were made to eliminate those pigments producing the greatest interference. The two methods described below proved satisfactory.

Method 1.—To 10 cc. of filtered sow urine, 1.25 cc. of 10% aqueous $ZnSO_4$ and 1.25 cc. of N/2 NaOH were added. The solution was then thoroughly mixed, and centrifuged for five minutes or filtered, Whatman No. 40 paper being used. The clear liquid is then substituted for urine in step 1 of the Cuboni procedure.

Method 2.—After 10 cc. of filtered sow urine was hydrolyzed with 2 cc. of concentrated HCl, 0.5 Gm. of sodium sulfite was added. The material was then thoroughly mixed, allowed to stand for 10 to 15 minutes, cooled in an ice-water bath and filtered through qualitative paper. The filtrate thus obtained was then treated as described in step 3 of the Cuboni method.

The two methods are equally effective in removing interfering pigments from sow urine, but the gas which is liberated in the sodium-sulfite method necessitates the use of a hood. In addition, the zinc-sulfate method is more rapid.

When either method for eliminating interfering pigments from sow urine is used, additional pigments may be removed by filtering, following cooling in step 3 of the Cuboni procedure, as shown in figure 1.

Four solvents—benzene, chloroform, toluene and carbon tetrachloride—were compared for extracting estrogens from the urine. Each of these solvents was satisfactory, but the green fluorescence was brighter when chloroform was used. This was particularly true if the interfering pigments were not removed.

Sterols also give a green fluorescence with H_2SO_4 similar to that produced by the estrogens in this test. Because of this, positive tests may occasionally occur in the urine of nonpregnant animals. As a confirmatory test, the addition of 0.5 to 1 cc. of H_2O (Kober[5]) to the completed tests (fig. 1, step 6) removes the green fluorescence caused by all substances except the estrogens. This additional step makes the test specific for estrogens and allows an accurate distinction between doubtful and positive pregnancy tests in swine. Where chloroform is used to extract the estrogens, the addition of water may cause a portion of the acid mixture to rise to the top of the solution. If this should occur, some difficulty in reading the confirmatory test may be experienced.

The Cuboni[6] test as described for the mare

*From the Department of Animal Husbandry, Missouri Agricultural Experiment Station (journal series paper No. 754) and the Bureau of Animal Industry, U. S. Department of Agriculture.

[1]Faiermark, S., and Bigos, V.: The hormonic method of early pregnancy diagnosis in the sow. (Title trans.) Probl. Zhivotn., i (1933), pp. 73-75; Anim. Br. Abst., iv (1936), p. 284.

[2]Faiermark, S.: Hormone pregnancy diagnosis in the sow. (Title trans.) Probl. Zooteh. eksp. Endokrin., ii (1935), pp. 48-58; Anim Br. Abst., iv (1936), p. 62.

[3]Kust, D., and Struck, M.: Secretion of sex hormones in the urine and blood of pregnant sows. (Title trans.) Deut. Tierarztl. Wochnschr., xliii (1934), pp. 54-56; Anim. Br. Abst., iii (1935), p. 158.

[4]Cuboni, E.: A simple and rapid chemical hormonic pregnancy diagnosis. (Title trans.) Klin. Wochnschr., xiii (1934), pp. 302-303; Anim. Br. Abst., ii (1934), p. 5.

[5]Kober, S.: Biochem. Z., ccxxxix (1931), pp. 209-212.

[6]Cuboni, E.: Rapid pregnancy diagnosis in the mare. (Title trans.) Clin. Vet., lvii (1934), pp. 85-93; Anim. Br. Abst., iv (1936), p. 284.

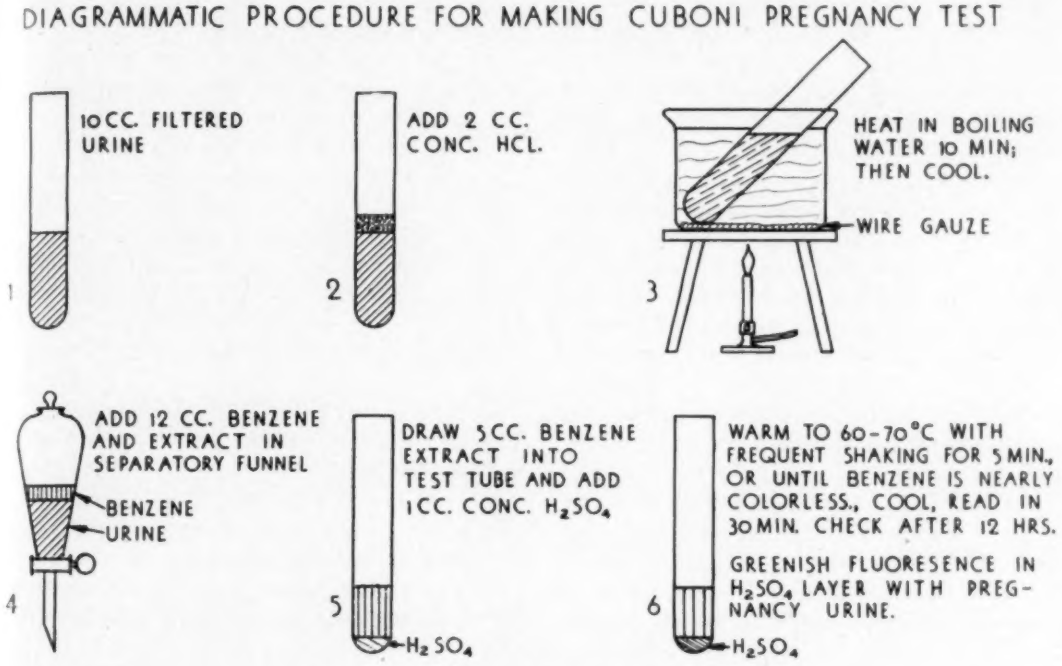
DIAGRAMMATIC PROCEDURE FOR MAKING CUBONI PREGNANCY TEST
10 CC. FILTERED URINE
1
ADD 2 CC. CONC. HCL.
2
HEAT IN BOILING WATER 10 MIN; THEN COOL.
WIRE GAUZE
3
ADD 12 CC. BENZENE AND EXTRACT IN SEPARATORY FUNNEL
BENZENE
URINE
4
DRAW 5 CC. BENZENE EXTRACT INTO TEST TUBE AND ADD 1 CC. CONC. H_2SO_4
5
H_2SO_4
WARM TO 60-70°C WITH FREQUENT SHAKING FOR 5 MIN., OR UNTIL BENZENE IS NEARLY COLORLESS., COOL, READ IN 30 MIN. CHECK AFTER 12 HRS.
GREENISH FLUORESCENCE IN H_2SO_4 LAYER WITH PREGNANCY URINE.
6
H_2SO_4

Fig. 1. Procedure for chemical pregnancy test.

(fig. 1) with the additional modifications given above was used in all experiments on the sow.

During the fall of 1940, seven sows were selected as experimental animals and one of these sows was bred each day for seven consecutive days. Seven days after the first sow was bred and at seven-day intervals thereafter, urine specimens were secured. Thus, we were provided with specimens of sow urine from this group representative of each day of the entire gestation period in the sow. However, one sow failed to settle and was, therefore, used as a negative control along with twelve other nonpregnant females. From the six sows which subsequently farrowed, a total of 84 samples were tested along with a similar number of samples from the nonpregnant control group.

As urine was more easily obtained from the sow early in the day, the first sample voided in the morning was used in each of the above cases.

RESULTS

A definite correlation was observed between the periods of maximum estrogen excretion reported for the sow and the periods during which positive tests were obtained in all pregnancy urine samples. This correlation is shown in figure 2. Figure 2 also shows that in every case all tests were positive from the twentieth to the thirty-third day of gestation and from the seventy-second day to parturition. During the interval from the thirty-second to the seventy-second day, a period of minimum estrogen excretion, all tests gave negative results.

Urine specimens from the one sow that failed to settle and from the twelve other

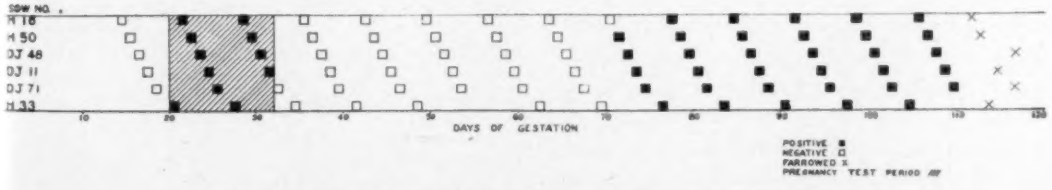

Fig. 2. Results of pregnancy tests throughout gestation in swine.

negative controls gave a negative result in every case.

All of the sows were either Hampshire or Duroc Jersey. No differences could be detected in the results of tests on these breeds.

Breeding dates are essential for proper use of this test in diagnosing pregnancy in the early part of the gestation period because a positive test is obtained only from 21 to 32 days (inclusive) in the first half of pregnancy. One will have few occasions for using the test to diagnose pregnancy during the latter part of gestation because external pregnancy symptoms will usually be evident at this time.

SUMMARY AND CONCLUSIONS

The data presented are indicative of a correlation between the periods of maximum estrogen excretion in the pregnant sow and the periods during which positive tests were obtained. Negative pregnancy tests were obtained during the periods of minimum estrogen excretion and on all urine samples from nonpregnant sows.

Our results in addition show that the modified Cuboni test as used in obtaining the data enables one to detect pregnancy early in the gestation period in the sow and to clearly differentiate the pregnant from the nonpregnant animal.

Therefore, the authors recommend the modified Cuboni technic as described below in detail and with each step in its proper sequence for the diagnosis of pregnancy in the sow:

1) To 10 cc. of filtered sow urine add 1.25 cc. of 10 per cent aqueous $ZnSO_4$ and 1.25 cc. of N/2 NaOH. Mix thoroughly.

2) Centrifuge or filter to remove precipitated material.

3) Add 2 cc. of concentrated HCl to the filtrate or supernatant fluid and hydrolize for ten minutes. (See figure 1.)

4) Cool to approximately 15 C. and filter.

5) Add 12 cc. of benzene (or other solvent) to the filtrate in a separatory funnel and shake for three to five minutes.

6) Allow the mixture to stand until the urine and the solvent have completely separated. (If separation is extremely slow, the addition of a small amount of Na_2CO_3 is an aid.)

7) Drain 5 cc. of the solvent extract into a clean, dry test tube. Add 1 cc. of concentrated H_2SO_4.

8) Heat to 60 to 70 C., with frequent shaking, for five minutes, preferably until the solvent is nearly colorless.

9) Cool thoroughly and allow to stand for at least 30 minutes before reading the test.

10) Use reflected sunlight, or blue artificial light in a dimmed room, for illumination in reading the test.

11) A green fluorescence of the acid mixture indicates a positive test. A negative result is indicated by a brown or reddish brown color.

12) Check results by adding 1 cc. of H_2O to the mixture after it has stood for twelve hours. Shake thoroughly and cool. All indications of green will be absent from the nonpregnant samples after the water is added.

13) The test must be run from the twenty-first to the third-second day of gestation (fig. 2); therefore, breeding records are essential.

Some Factors Affecting Fertility in Swine*

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STERILITY AND LOW fertility are important causes of loss to the livestock producer, and improvement in the level of fertility should be an important aim of the breeder coincident to improvement in the quality and efficiency of his animals in other respects.

The extent to which reproductive failures occur in various classes of livestock has been reviewed by Phillips[1] and the possible causes of these failures have been discussed; hence, these will not be dealt with here except to point out that the normal course of the reproductive process may be interrupted at any of several points owing to (1) failure to form spermatozoa or ova, or a reduction of the number or quality of germ cells produced, (2) failure of animals to copulate owing to lack of sexual desire or injury, (3) death of the liberated ova or spermatozoa before fertilization, (4) failure of fertilization, (5) failure of implantation in the uterus, (6) failure of normal embryonic and fetal development, and resorption or abortion, and (7) stillbirth at the end of a normal term. Little information is available on the relative importance of the various causes of reproductive failures, and for swine, few figures have been recorded in the literature on the incidence thereof.

The present study represents an attempt to determine the incidence of reproductive failures in swine and to obtain some information on the importance of various factors which may be responsible for them.

MATERIAL

Data used in this study were obtained from the experimental herd at the U. S. Animal Husbandry Experiment Station, Beltsville Research Center, Beltsville, Maryland. The numbers of individuals involved, the numbers of seasons in which sows were bred, and the years during which data were obtained on each breed are shown in table I.

TABLE I—Summary of Material

BREED	YEARS (INCL.)	NUMBER		BREEDING SEASONS*
		BOARS	SOWS	
Chester White	1926–38	57	78	318
Duroc	1926–38	23	64	173
Landrace	1934–38	32	53	174
Poland China	1926–38	66	135	513
Tamworth	1926–33	36	30	111
Yorkshire	1929–38	19	22	65
Total		233	382	1,354

*Each breeding season in which a sow was mated is included as one item.

DATA AND DISCUSSION

The 382 sows included in this study were exposed to breeding in a total of 1,354 breeding seasons. From this number, 1,058 gestations resulted, and at 296 sow breeding seasons conception failed to occur. This means that in this group of sows conception failed in 21.9 per cent of the cases, or that more than one fifth of the sows exposed to breeding failed to conceive. The data are summarized by breeds in table II, along with data on other points.

Examination of the data on the number and percentage of breeding seasons at which conceptions failed to occur indicates that differences exist in the breed groups under observation, the range being from 13.9 per cent in the Duroc to 36.9 in the Tamworth. The average number of services required per pregnancy also varies in the different breed groups, and there is a direct relationship between the percentage of breeding seasons at which conception failed and the number of services required per pregnancy. To a certain extent, the sizes of these two figures are, no doubt, measures of the same factors which acted to reduce fertility.

*From the Animal Husbandry Division, Bureau of Animal Industry, U. S. Department of Agriculture.

[1]Phillips, R. W.: Reproductive failures in livestock. USDA Yearbook (1939), pp. 476-482.

TABLE II—Summary of Fertility of Sows in Various Breeds

Breed	Sow Breeding Seasons		Per Cent Breeding Seasons at Which Conception Failed	Average Number Services per Pregnancy	Total Number Pigs Born	Average Number Pigs per Litter	Pigs Weaned		Average Per Cent Abnormal Pigs
	Resulting in Pregnancy	Not Resulting in Pregnancy					Number	Per Cent	
Ch. White	254	64	20.1	1.60	2,108	8.29	5.46	65.7	5.41
Duroc	149	24	13.9	1.28	1,415	9.50	5.96	62.7	4.31
Landrace	147	27	15.5	1.46	1,460	9.93	6.33	63.8	5.34
Pol. China	385	128	25.0	1.64	2,656	6.90	4.42	64.1	3.76
Tamworth	70	41	36.9	1.99	494	7.06	4.47	63.4	4.85
Yorkshire	53	12	18.5	1.57	582	10.98	7.51	68.4	5.84

Definite differences also appear in the size of litter, with a range of from 6.9 in the Poland China to 10.98 in the Yorkshire. Some differences also appear in the percentage of pigs born that are raised to weaning age.

The figures on abnormal pigs include normally formed stillborn pigs, recognizable mummies and deformed pigs. Here, also, there is some indication of differences among the groups observed, the range being from 3.76 to 5.84 per cent. In all breeds, 8,715 pigs were born and 411, or 4.72 per cent, were classified as abnormal and represent a source of loss to the producer. Of this number, 404 were classed as stillbirths. Studies by Hammond[2] and Corner[3] indicate that only about 70 and 67.4 per cent, respectively, of the ova shed by sows are represented by normal fetuses. Their data were from sows slaughtered prior to parturition; hence, all or most of the pigs classified in the present study under "stillbirths" would have appeared as normal fetuses. Thus, it seems that in addition to the prenatal loss, there is a loss of about 4.7 per cent of the remainder at the time of birth. Approximately 35 per cent of the pigs born were not alive at weaning.

Data presented in table III indicate that there is a considerable loss of ova due to infertile matings. In the group of sows which farrowed there were 197 infertile matings, and in the sows not farrowing there were 409, or a total of 606 infertile matings as compared with 1,058 fertile matings. If we consider that the loss in this herd from ovulation to parturition is the same as that observed by Hammond[2] and Corner[3] (an average of 31.3 per cent), we can determine the fate of ova shed. The

TABLE III—Proportion of Sows Bred 1, 2, 3 and 4 Times Within a Breeding Season

Breed	Number Bred			
	1 Time	2 Times	3 Times	4 Times
Sows Farrowing				
Ch. White	212	28	13	1
Duroc	135	14	...	...
Landrace	123	20	3	1
Pol. China	329	44	11	1
Tamworth	57	9	4	...
Yorkshire	45	6	1	1
Total	901	121	32	4
% Bred	85.2	11.4	3.0	.4
Sows Not Farrowing				
Ch. White	41	17	4	2
Duroc	22	1	1	...
Landrace	19	5	3	...
Pol. China	90	29	6	3
Tamworth	31	9	1	...
Yorkshire	7	3	2	...
Total	210	64	17	5
% Bred	71.0	21.6	5.7	1.7

[2]Hammond, J.: Further observations on the factors controlling fertility and foetal atrophy. J. Agr. Sci. (England), xi (1921), pp. 337-366.

[3]Corner, G. W.: The problem of embryonic pathology of mammals, with observations on intra-uterine mortality in the pig. Am. J. Anat., xxxi (1923), pp. 523-545.

TABLE IV—Fate of Ova Shed by Sows at Estrous Periods in Which They Were Mated

Fate of Ova	In Sows Which Produced Litters (%)	In All Sows Bred (%)
Loss from failure to conceive	....	36.4
Loss during gestation	31.3	19.9
Loss at parturition	3.2	2.0
Loss from birth to weaning	22.9	14.6
Live pigs at weaning	42.6	27.1
Total	100.0	100.0

figures showing the fate of ova are presented in table IV. These are estimates, of course, based upon the only information available on prenatal mortality and upon actual data on other points contained in the large volume of data at hand. They can not be considered as absolute values for all conditions, but are of considerable importance in indicating the phases in the reproductive process where failure is most apt to occur.

When one considers that only 27.1 per cent of the ova which might develop into weanling pigs actually reach this stage, it is obvious that there is room for improving the level of fertility. Figures presented in table IV indicate that infertile matings rank first as the cause of failures, that losses from conception to parturition rank second and those from parturition to weaning third.

The data at hand can not be analyzed to determine the degrees to which the sow and the boar, respectively, are responsible for losses due to infertility. However, some observations of interest were made on variations in the level of fertility of boars. A total of 91 boars in all breeds were each bred to five or more sows. The percentages falling into various groups on the basis of services required per pregnancy were as follows:

Services per Pregnancy	*Per Cent*
1.0-1.4	46.1
1.5-1.9	28.6
2.0-2.9	18.7
3.0-3.9	2.2
4.0-4.9	1.1
5.0-up	2.2
No pregnancies	1.1

In the same group of boars there was considerable variation in the average size of litters produced, the percentages in each group on the basis of litter size being as follows:

Size of Litter	*Per Cent*
1 - 2.4	1.1
2.5- 4.9	1.1
5.0- 7.4	41.1
7.5- 9.9	38.9
10.0-12.4	14.5
12.5-14.9	3.3

Variations in the average percentage of pigs weaned in litters sired by each boar also were quite marked in this group, the figures being as follows:

Range in Average Percentage of Pigs Weaned	*Per Cent in Each Group*
21- 30	1.1
31- 40	4.5
41- 50	10.0
51- 60	21.1
61- 70	31.1
71- 80	20.0
81- 90	10.0
91-100	2.2

Variations also were observed in the average percentage of abnormal pigs in the litters sired by each of these boars. The percentages are given below:

Range in Average Percentage of Abnormal Pigs	*Per Cent in Each Group*
None	22.2
0.1- 5.0	45.6
5.1-10.0	20.0
10.1-15.0	8.9
15.1-20.0	1.1
20.1-25.0	1.1
25.1-27.5	1.1

Since matings were usually made on the basis of characteristics other than level of fertility, it hardly seems feasible that all of the variation observed could be accounted for by the fertility level of sows with which individual boars were mated. Rather, it appears that the boars must have been partially responsible.

The level of fertility in this herd is not as high as could be desired. One factor that may be important is the presence of brucellosis. A summary of results of tests for this disease, from 1930 to 1937, is given in tables V and VI. The number tested includes each animal once for each year

TABLE V—Results of Tests for Brucellosis in Boars

Breed	No. Boars Tested	Reactors	
		No. Pos. 1:50–1:1,000 Dilution	No. Susp. 1:25 Dilution
Ch. White	54	12	8
Duroc	19	3	4
Landrace	39	0	2
Pol. China	62	25	6
Tamworth	25	10	3
Yorkshire	11	0	0
Total	210	50	23

TABLE VI—Results of Tests for Brucellosis and the Incidence of Abortions in Sows

Breed	No. Sows Tested	Reactors		No. Actual Abortions
		No. Pos. 1:50–1:1,000 Dilutions	No. Susp. 1:25 Dilutions	
Ch. White	99	38	12	13
Duroc	71	15	13	3
Landrace	87	11	13	1
Pol. China	188	84	26	13
Tamworth	34	11	2	5
Yorkshire	23	2	3	0
Total	502	161	69	35

in which a blood test was made. If tested more than once in one year, it is included only once. If results for these tests differed, only the test most indicative of disease is included. Obviously, this method of listing gives the maximum possible amount of disease present, as indicated by the blood tests. A total of 35 abortions were observed. Over one half of these abortions as well as over one half of the positive tests occurred within one year (1933).

The incidence of positive reactions to the test for brucellosis is sufficiently high to indicate that the presence of this disease may have been one of the important factors in the low reproductive level, entirely apart from actual abortions. It is also possible that brucellosis might have affected the reproductive process at some points more than at others. If this is true, the relative proportions of the ova lost at different stages might not be representative of conditions in a disease-free herd.

Other studies of this type, utilizing breeding records from herds that are free from brucellosis, are needed. If studies were made of herds that have had a high incidence of that disease for a few years, after which it has been possible to eliminate it, then records accumulated on the disease-free herds for a period of time would be extremely interesting.

SUMMARY

1) There appear to be differences in the level of fertility in the breed groups observed, as measured by proportion of breeding seasons at which conception failed, number of services required per pregnancy and the size of litter. Differences also were found in the proportion of abnormal pigs and the proportion of pigs weaned. These apparent differences in breed groups would not be generally applicable to the breeds as a whole.

2) The relative importance of points at which the reproductive process fails, as indicated by the percentages of ova lost, are as follows: (a) failure of conception, 36.4 per cent, (b) loss from conception to parturition, 19.9 per cent, (c) loss from parturition to weaning, 14.6 per cent, and (d) loss at parturition, 2 per cent. This leaves 27.1 per cent of the ova which have the chance to be fertilized represented by live pigs at weaning.

3) The level of fertility in boars appears to be quite variable, as indicated by services required per pregnancy, size of litter sired, percentages of offspring weaned and proportion of abnormal pigs sired.

4) The possible relationship of brucellosis to the reproductive level of the herd is discussed.

5) The need for further studies on other herds is pointed out.

The Chemotherapy of Infectious Coryza with Sulfathiazole: Correlation Between Feeding Levels and Blood Concentrations*

FRANK S. SCHLENKER, B.S., M.S., JOHN P. DELAPLANE, D.V.M., M.S., and H. O. STUART, B.S., M.S.

Kingston, R. I.

RECENTLY Delaplane and Stuart[1] found that sulfathiazole used in the feed was of value in preventing the development of coryza in adult cockerels exposed to inoculations of virulent organisms of *Hemophilus gallinarum* and that if given to chickens showing symptoms of the disease, it hastened their recovery. As long as the sulfathiazole feeding was maintained, no symptoms of the infection were apparent, but after it was discontinued, the birds developed the disease, indicating that the organisms were able to survive for a period of at least two weeks, but did not grow in sufficient numbers to produce clinical evidences. They also found that the blood from birds fed sulfathiazole retarded the growth of *H. gallinarum* in cultures.

These feeding results gave rise to a number of questions concerning feeding technics that could not be answered without a complete understanding of the blood chemistry of treated birds.

METHODS

The sulfathiazole content of blood was determined by coupling with N(1-naphthyl) ethylenediamine dihydrochloride at pH 1.0-2.0.[2] Recovery experiments were made by adding known amounts of sulfathiazole to 2 cc. of citrated blood to which 40 cc. of water was added and either sodium tungstate solution and 6 N H_2SO_4 or 15 per cent trichloracetic acid to bring the final volume to 50 cc. The amounts of sulfathiazole added were 0.04 mg. to 0.40 mg. per 50 cc. in increments of 0.04 mg. All determinations were made with an ocular colorimeter.

The results shown in table I indicate that trichloracetic acid should be used in the preparation of blood filtrates. Wherever possible, aliquots were taken so as to insure a final concentration of 0.2 to 0.4 mg. of sulfathiazole per 50 cc.

There is apparently no normally occurring blood constituent that reacts with the reagents involved in the sulfathiazole determination, since a number of trials were made on normal blood and no color could be detected.

TABLE I—Percentages of Sulfathiazole Recovered from Whole Blood

Sulfathiazole Concentration (Mg./50 cc.)	Sodium Tungstate +6 N H_2SO_4	15% Trichloracetic Acid
0.04—0.16	42.0	113.0
0.20—0.40	43.0	100.5

Blood samples were taken from the wing veins and collected in 2 cc. of 2 per cent sodium citrate in normal saline solution. The sulfathiazole was fed either in gelatin capsules or in regular mash. The first method was employed when definite amounts per kilogram (2.2 lb.) of body weight were desired, and the second, under practical poultry-feeding conditions. The experimental data are presented in two ways. When sulfathiazole was administered orally, the results for each of the four birds used per experiment are given singly. In those instances where the drug was incorporated in the mash, however, each experimental value is the average of individual determinations obtained from four birds. In some instances the variation between maximum and minimum figures is less than 0.1 mg. per cent; in others, it is as much as 1.2 mg. per cent. This extreme variation is rare and the difference is usually in the order of 0.2 to 0.4 mg. per cent.

FEEDING EXPERIMENTS

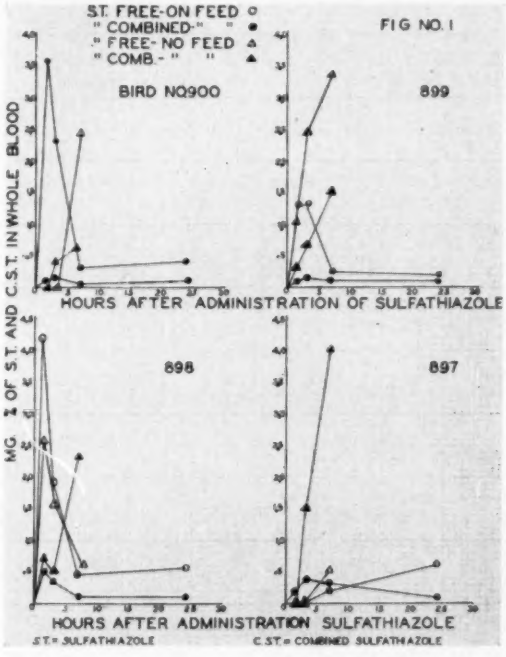
Two sets of four birds each were fed, by capsule, at the rate of 0.2 Gm. of sulfathiazole per kilogram of body weight. Each set consisted of two Rhode Island Red and two White Leghorn cockerels. Their individual weights varied from 4 to 9 lb. The

*Contribution No. 595 of the Rhode Island Agricultural Experiment Station.

[1]Delaplane, J. P., and Stuart, H. O.: The chemotherapeutic value of sulfathiazole in preventing and treating infectious coryza in chickens. (In press, J.A.V.M.A.)

[2]Bratton, A. C., and Marshall, E. K., Jr.: A new coupling component for sulfanilamide determination. J. Biol. Chem., cxxviii (1939), pp. 537-550.

weighed amounts of sulfathiazole were fed under two conditions: one in which the birds had access to mash before and during the absorption period, the other in which it was taken away the night before and withheld for the duration of the experiment. The two sets of cockerels were given the drug under both of these conditions.

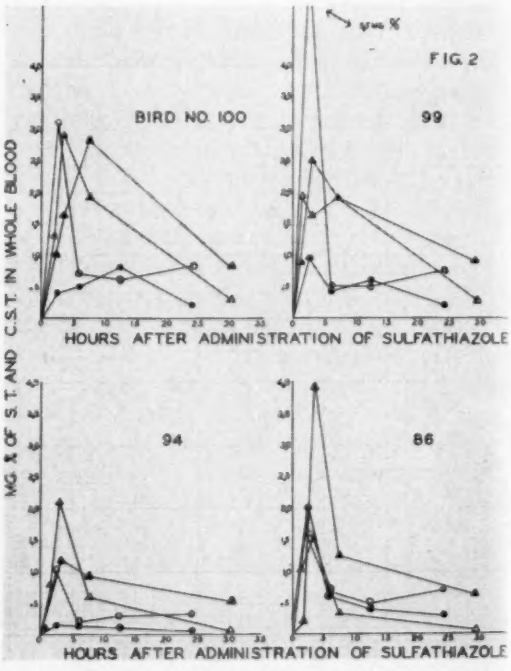


Blood samples were taken frequently in order to ascertain the rapidity and duration of absorption of sulfathiazole as reflected by blood levels. The data derived from this duplicate experiment are presented in figures 1 and 2.

Considering first the experiment during which the birds were "on feed," it is seen that absorption is rapid and free sulfathiazole reaches its peak concentration between 1½ and 3 hours after oral administration.

A rapid drop then occurs after which the drug gradually disappears from the blood, some time after 30 hours. There appears to be a tendency for free sulfathiazole to increase slightly after dropping from its peak concentration. If it is assumed that the ingested free form of sulfathiazole has been largely eliminated, then this rise may be at the expense of the combined form, as suggested by its continuous decrease. Combined sulfathiazole is present only in small amounts; it reaches a peak at approximately the same time as the free form and it disappears after 25 hours. This general picture failed to be true in only two instances. The blood picture of bird 86 shows the rapid rise and fall as stated, but combined sulfathiazole reached a higher peak than the free form, although after this it followed the general relationship of these two types. With bird 897, the results were contrary to expectations until the fourth sample. No explanation can be offered for this discrepancy.

The blood picture is different when sulfathiazole is administered in the absence of



feed. Under the conditions set up by this factor the peak concentration of the free form is reached in 1.5 to 7 hours after feeding. Furthermore, there is no apparent relationship between the free and combined sulfathiazole. It is to be noted that the combined form is in most cases present in concentrations far in excess of that in the blood from birds "on feed."

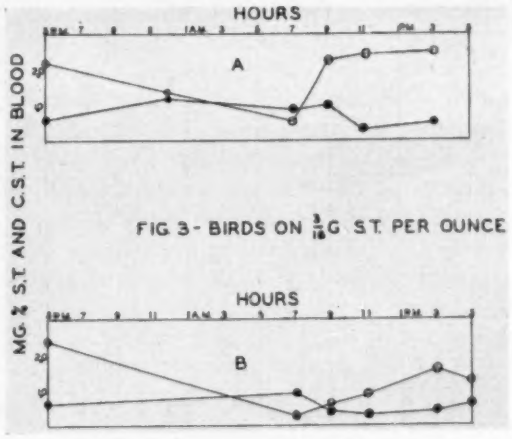
It is necessary, however, to keep the blood levels at a concentration sufficient to

control infection. This can be done by adding sulfathiazole to mash, feeding *ad libitum* and not supplementing with any other form of feed.

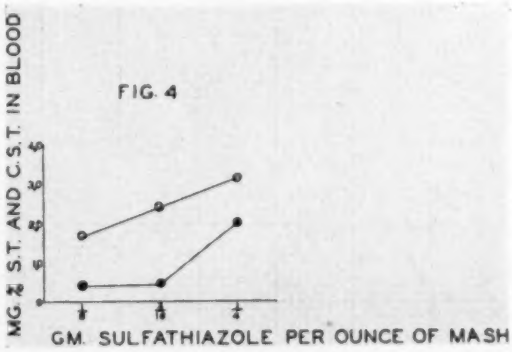
Preliminary feeding experiments indicated that birds inoculated nasally with *H. gallinarum* showed no symptoms when fed mash containing 1, ½, ¼ and 3/16 Gm. of sulfathiazole per ounce. When the sulfathiazole level was lowered to ⅛ Gm. per ounce, subclinical symptoms appeared. With these results as a basis, three levels have been studied: ⅛, 3/16 and ¼ Gm. sulfathiazole (99.9 per cent) per ounce of feed.

A study was made to ascertain variations in the sulfathiazole content of blood during a 24-hour period. In this experiment four cockerels (two Rhode Island Reds and two White Leghorns) were given access to mash containing 3/16 Gm. of sulfathiazole per ounce. The results are shown in figure 3A and indicate a level of 2.4 mg. per cent during the late afternoon. This concentration gradually decreases until a minimum is reached just prior to the first feeding period. After this the blood concentration of free sulfathiazole rises to 2.2 mg. per cent and becomes established at a 2.4 mg. per cent level. Combined sulfathiazole appears to have an inverse relationship to the free form. This experiment was repeated during a period of low daily temperature (0 to 10 F.); the data are given in figure 3B. These figures show the correlation between feeding habits and sulfathiazole content of the blood. Food consumption was low and, consequently, the amount of sulfathiazole available for absorption was at a minimum. Instead of a sharp rise, such as occurred during a day of favorable temperature conditions, in this instance the rise was gradual, and reached a maximum of only 1.6 mg. per cent. During this cold period other birds not on test likewise showed a low mash intake. The relation between the combined and free form is similar to that of the previous experiment.

A comparison of the three feeding levels as reflected by blood concentrations indicates a direct proportionality between these



two factors at the concentrations studied. This is apparently not true for the combined sulfathiazole, as shown in figure 4. It should be emphasized that under certain conditions this regular increase in blood sulfathiazole with increasing levels of the drug in feed might not occur, inasmuch as many factors contribute to the processes of intake and absorption. Environmental factors, such as length of day, temperature and sulfathiazole content of feed, will play an important part in determining the slope of this curve.



DISCUSSION AND SUMMARY

From a study of eight cockerels given sulfathiazole at the rate of 0.2 Gm. per kilogram of body weight, it is apparent that the absorption of sulfathiazole is rapid. A peak concentration in blood is reached within 1½ to 3 hours after oral administration when the birds are "on feed." An immediate drop takes place, and then a slight rise, after which the residual amounts

of the drug are eliminated. This regularity is forestalled when the drug is given in the absence of mash. No reason can be offered for these differences. The results indicate that from the standpoint of practicability, it is necessary to keep sulfathiazole before the birds at all times in order to establish a constant effective level of the drug in the blood.

A concentration of 3/16 Gm. of sulfathiazole per ounce of mash or greater (¼, ½ and 1 Gm.), when fed *ad libitum*, established a blood concentration which prevented the development of symptoms of coryza in artificially inoculated birds. When the drug level was lowered to ⅛ Gm. per ounce, however, subclinical symptoms appeared. It is of interest to note that at a 3/16-Gm. level, a maximum blood concentration of 2.4 mg. per cent was established, whereas at the ⅛-Gm. level, this concentration was only 1.6 mg. per cent. These results appear to be correlated with those obtained from cultural methods.

It is possible that this lower level would prove effective in preventing the disease through contact exposure, because of the great difference between the number of organisms used to inoculate the birds artificially and the relatively small number which would normally be present from natural exposure.

From a practical standpoint the effective use of sulfathiazole would have to be based on knowledge concerning the status of the infection in each individual flock.

The Autonomic Innervation of the Female Genitalia in the Domestic Fowl and Its Correlation with the Aortic Branchings*

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Columbus, Ohio

DESPITE THE RAPID increase in the importance of the poultry industry, there is little, if any, literature dealing adequately with the nervous system of the common fowl.

With this fact in mind it was decided to determine, if possible, the innervation of the female genitalia, inasmuch as this group of organs is economically important in the hen.

It was observed that the abdominal nerve plexuses were intimately correlated with the aortic branchings and that, therefore, these branchings should be included in a study of this subject.

MATERIALS AND METHODS

The ages of the birds varied from 48-hour embryos to mature laying hens. Serial sections were made of the embryos and immature fowls and the mature ones were examined by microdissection.

A colored vinyl resin was injected into the arterial system to help differentiate the nerve tissue and facilitate the determination of the aortic branchings.

Several other species of birds were examined, but no outstanding differences from the structures of the hen were found.

INNERVATION

The innervation of the female genitalia was traced mainly by the use of vinylite injection mass. This mass filled the blood vessels and made it easier to trace the nerves and differentiate them from the vascular system.

The innervation of the ovary was first investigated. It was found that there is a large ovarian plexus located just above the ovary (fig. 1). The location of this plexus varies in different birds. However, it was most often found dorsal and posterior to the middle of the ovary, on the left ventral portion of the vertebral column just posterior to the vertebral end of the sixth rib. The fibers from this plexus follow the ovarian artery and penetrate the gland at the same level as the vessel, although some may enter directly from the primary plexus. This plexus is connected by anastomosing fibers with the coeliac plexus anteriorly and the adrenal, aortic and anterior mesenteric plexuses posteriorly.

In addition to supplying the ovary with nerve fibers, the ovarian plexus also sends some fibers to the anterior portion of the oviduct, notably to the infundibulum. These fibers leave the posterior portion of the plexus and enter the anterior portion of the dorsal ligament. They reach the wall of the oviduct by traversing the width of the dorsal ligament.

The extrinsic nerve supply to the oviduct was found to be derived from various abdominal plexuses, all of which are a part of the autonomic system and indirectly of the sympathetic system. As previously stated, the anterior extent of the oviduct (the infundibulum) is supplied with fibers originating in the posterior part of the ovarian plexus. The remainder of the oviduct is innervated by fibers arising from the various autonomic plexuses located along the abdominal aorta.

The renal plexus (fig. 1), located at the origin of and interlacing the renal arteries, gives most of its fibers to the kidneys. However, some of these fibers run in a ventral direction, enter the dorsal ligament and are distributed to both the suspensory tissue and the oviduct. The left and right renal plexuses are connected by anastomosing fibers, giving the appearance of a single, large plexus. If it is desired to differentiate between these two sections, it should be noted that the innervation of the oviduct supplied by this plexus comes only from the

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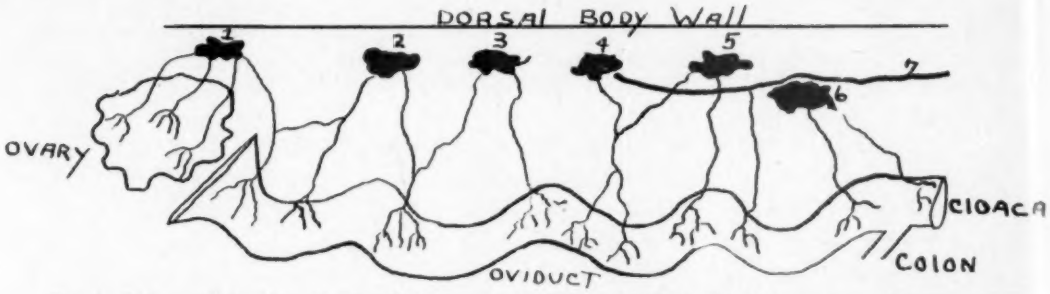

Fig. 1. Schematic drawing, showing the innervation of the genitalia in the common hen. (1) Ovarian plexus. (2) Left renal plexus. (3) Aortic plexus. (4) Sciatic plexus. (5) Posterior mesenteric plexus. (6) Pelvic plexus. (7) Left sympathetic trunk.

left side, or left renal plexus. The middle portion of the oviduct is supplied with fibers originating in the abdominal aortic plexus (fig. 1). The exact location of this aortic plexus varies. However, it is usually located on the ventral surface of the posterior aorta between the renal and sciatic arteries. The fibers from this plexus also penetrate the dorsal ligament, supply this structure and have their endings in the wall of the gland.

There is a plexus located at the origin of each sciatic artery (fig. 1). In one specimen dissected, a fiber tract was traced from the left sciatic plexus into the dorsal ligament of the oviduct. In subsequent specimens, however, the presence of this tract could not be proved.

At the origin of the posterior mesenteric artery the distinct posterior mesenteric plexus was located (fig. 1). It was found that the usual location of this plexus is on the ventral surface of the aorta just behind the origin of the posterior mesenteric artery. From this plexus small fiber bundles enter the dorsal ligament, traverse and innervate this structure, and have their terminations in the wall of the gland in the shell-membrane area. The posterior mesenteric plexus is connected in front with the sciatic and aortic plexuses and behind with the pelvic plexus.

The position of the pelvic plexus (fig. 1) was found to vary in different specimens. This plexus is a direct continuation of fibers from the posterior mesenteric and sciatic plexuses. There are two pelvic plexuses in the chicken, one on each side. Their usual location was found to be on the visceral wall of the pelvis approximately one inch below and lateral to the origin of the internal iliac arteries. Fibers leaving this plexus supply the posterior portion of the oviduct, the cloaca and the pelvic viscera. In reaching the oviduct the fibers which emanate from the pelvic plexus follow a path similar to that of the fibers from the preceding plexuses; that is, they penetrate the dorsal ligament and terminate in the wall of the gland.

In the lumbosacral region the left sympathetic trunk passes the origin of the posterior division of the lumbosacral plexus. At this area the trunk receives additional fibers from the cerebrospinal system and enlarges considerably. This enlargement of the trunk is in evidence for the remainder of its course. From the area of the lumbosacral plexus backward, the sympathetic trunk relinquishes its close attachment to the vertebral column. It runs in the extreme upper portion of the dorsal ligament of the oviduct and upon reaching the posterior part of the pelvic cavity it penetrates the lateral wall in the cloacal region and terminates in the same area as the internal iliac artery. At various points along its path this part of the trunk was found to give off fibers directly. These fibers drop ventrally and enter the dorsal ligament of the oviduct.

From the foregoing it is seen that the extrinsic nerve supply to the genital organs is obtained from abdominal and pelvic plexuses of the sympathetic system and the trunk itself. The only possible exception

is the cloaca. This structure is partially innervated by the sympathetic system through the pelvic plexus, but it also receives some afferent fibers from the sacral and coccygeal nerves. However, this structure is common to both the digestive and urogenital systems and can not be classified as just a part of the genital tract.

Johnson[1] found the following facts in regard to the intrinsic nerve supply of the oviduct: The intrinsic plexuses of the oviduct itself are rich in fibers, but contain relatively few nerve cells. However, the plexuses of the cloaca are rich in both fibers and cells.

Aortic Branchings

After determining the intimate relation between the abdominal plexuses of the sympathetic system and the aortic branchings, it was considered necessary to include in this experiment a discussion of the arteries having their origin at the aorta.

This phase of the work was found to be of additional value when it was determined that there are several branches of the aorta in the common fowl that differ materially from those of mammals.

In the following discussion the facts given regarding the chief aortic branchings will be those determined from a cockerel. In the male bird the branches are more discernible due to the absence of the vertebral attachments of the ovary and oviduct.

A separate section will be devoted to a discussion of the blood supply to the ovary and oviduct in the hen.

1) *The coeliac artery* is usually the largest of the aortic branches. It is given off as a single vessel at the area of the vertebral end of the sixth rib. It has the following branches of its own:

a) Gastric (proventriculus).—A single vessel supplying the glandular portion of the stomach.

b) Gastrosplenic.—A larger vessel supplying twigs to the left side of the muscular stomach (gizzard) and a branch to the spleen.

c) Hepatic.—A large vessel supplying branches to the right and left lobes of the liver, two branches to the spleen and a branch to the right side of the gizzard which anastomoses with the gizzard branch from the gastric. This vessel also gives several small branches to the pancreas and a twig which supplies the terminal portion of the duodenum.

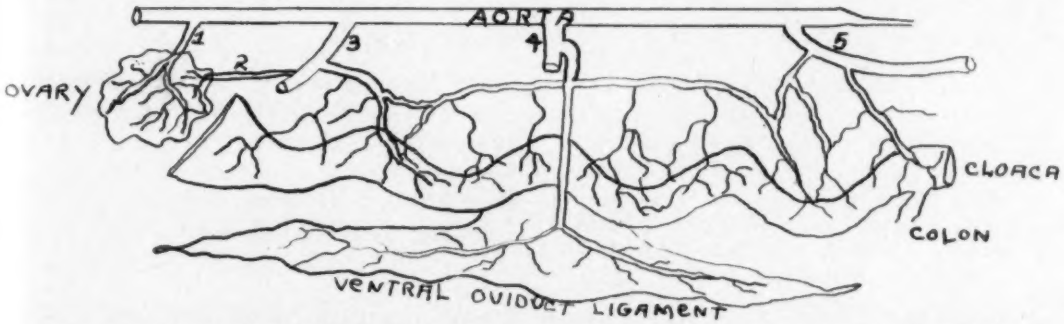

Fig. 2. Schematic drawing, showing the blood supply to the genitalia in the common hen. (1) Ovarian artery. (2) Ovarian branch of the left renal artery. (3) Left renal artery. (4) Left sciatic artery. (5) Left internal iliac artery.

2) *The intercostal arteries* are given off from the aorta at the level of the vertebral end of the corresponding rib. There are seven pairs of intercostal arteries; four of these are given off anterior to the coeliac and three posterior to it.

3) *The anterior mesenteric artery* is given off as a single vessel one-quarter inch posterior to the coeliac. It supplies the bulk of the small intestine and the ceca. The branch supplying the first part of the

[1]Johnson, J. S.: The innervation of the female genitalia in the hen. Abst., Anat. Rec., xxix (1925), p. 387.

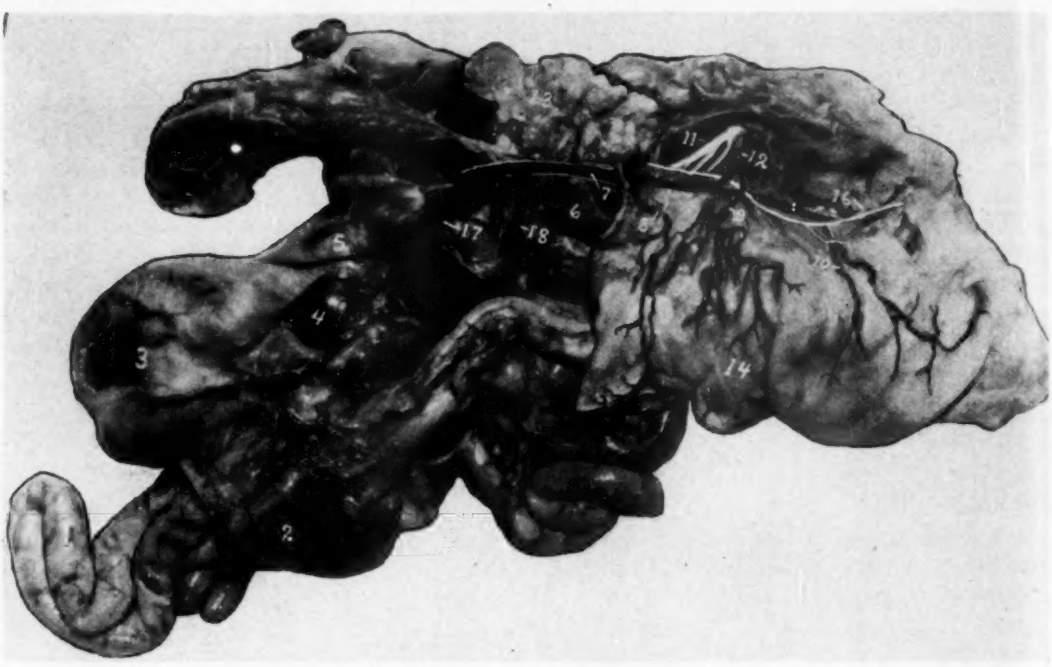

Fig. 3. Ventral view, showing the vertebral column and associated structures in a mature White Leghorn hen. (1) Duodenum. (2) Right cecum. (3) Muscular stomach (gizzard). (4) Spleen. (5) Glandular stomach (proventriculus). (6) Anterior lobe of right kidney. (7) Aorta. (8) Anterior oviduct artery. (9) Middle oviduct artery. (10) Posterior oviduct artery. (11) Posterior division of the lumbosacral plexus. (12) Left sciatic artery. (13) Ovary, pulled aside. (14) Oviduct. (15) Cloaca. (16) Posterior portion of left sympathetic trunk. (17) Coeliac artery. (18) Anterior mesenteric artery.

jejunum anastomoses with the duodenal branch of the hepatic.

4) *The spermatic arteries* are given off as paired vessels one-half inch posterior to the anterior mesenteric, just posterior to the vertebral end of the seventh rib. Each vessel gives its entire supply to the corresponding testicle.

5) *The renal arteries* are paired vessels given off one-quarter inch posterior to the spermatic. They supply the anterior lobe of the corresponding kidney. The left renal artery also gives a branch to the posterior part of the left testicle.

6) *The femoral arteries* are paired vessels given off one-half inch posterior to the renals. Each vessel divides, one inch from its origin, into two main branches. One branch penetrates the abdominal wall and traverses the anterior part of the thigh. The other runs downward and backward, gives twigs to the lateral abdominal muscles and terminates in the pelvic fascia and fat.

7) *The sciatic arteries* are paired vessels given off from the aorta in the area of a transverse plane through the acetabulum, about one inch posterior to the femoral arteries. Each artery passes through its corresponding pelvic wall between the pubis and ischium just posterior to the acetabulum and is the main arterial supply to the limb. Before penetrating the pelvic wall the left sciatic artery gives a branch to the posterior lobe of the left kidney. The right sciatic gives branches to both the middle and posterior lobes of the right kidney before penetrating the pelvic wall.

8) *The posterior mesenteric artery* is a single vessel given off from the ventral surface of the aorta one inch posterior to the sciatic origins. It soon divides into two branches, anterior and posterior. These

branches supply the colon. The posterior branch also sends some small twigs to the cloacal region.

9) *The internal iliac arteries* are paired vessels given off in the middle of the pelvic region three-quarters inch behind the posterior mesenteric. They represent, as in mammals, the bifurcation of the aorta. Each vessel accompanies the corresponding ureter, supplies it with blood, travels in a posterior direction and then is the main supply to the cloacal region. Twigs are given off along the way to the pelvic fascia and fat.

10) *The middle sacral* is the small continuation of the aorta. It continues on the ventral surface of the vertebral column and gives branches to the pelvic wall and surrounding tissue. It terminates in the coccygeal region and supplies the tail structures.

11) *The lumbar and coccygeal arteries* are given off from the posterior portion of the aorta and the middle sacral artery.

In every instance where the vessels originating from the aorta are paired, the right branch is given off anterior to the left. The distance between these trunks varied from one-sixteenth to one-quarter inch.

Blood Supply to the Oviduct

In the hen the following facts were noted in the genital blood supply. The arterial supply to the ovary is variable in different specimens. In most cases a large ovarian artery drops from the aorta just above the middle of the ovary and gives its entire supply to the ovary. The posterior part of the gland receives a small branch from the left renal. The variations noted in the ovarian blood supply include the following: In one bird two ovarian arteries were found to leave the aorta and penetrate the gland. In another one ovarian artery and two branches entered the posterior part of the ovary from the left renal.

The anterior portion of the oviduct (fig. 4), including the infundibulum, usually receives its blood from a branch of the left renal. This vessel leaves the renal before it penetrates the kidney. It enters the dorsal ligament, travels downward and backward in the ligament and branches repeatedly before terminating in the wall of the gland. In one specimen this supply to the anterior portion was found to come from a branch of the left femoral artery. This was considered, however, as just an atypical specimen and not as a generality.

The blood supply to the middle portion of the oviduct (fig. 4) comes from a branch of the left sciatic artery. This vessel leaves the sciatic a short distance from the aorta and immediately enters the dorsal ligament where it spreads out in a fan-shaped manner, supplies the ligament and terminates in the wall of the gland. One of the branches of this vessel crosses the left side of the oviduct and supplies the ventral ligament of the gland.

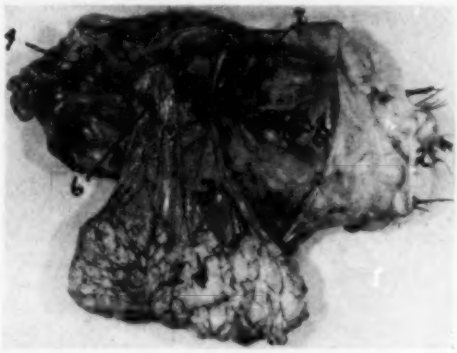

Fig. 4. Ventral view of an injected specimen, showing the trunk of the bird with attached oviduct. (1) Oviduct with injected arteries. (2) Cloaca. (3) Dorsal ligament of oviduct. (4) Ovary, pulled aside. (5) Left kidney. (6) Anterior oviduct artery. (7) Middle oviduct artery. (8) Posterior oviduct arteries.

The posterior portion of the oviduct (fig. 4) receives its blood supply from one or more branches of the left internal iliac artery. Usually two branches are present, but in some cases only one could be located. The first of these branches leaves the internal iliac about one inch from the aorta, the second slightly further back. These vessels supply blood to the posterior portion of the oviduct in the same manner as that used by the vessels supplying the middle and anterior portions, namely, by entering

the dorsal ligament, supplying it and terminating in the wall of the gland.

The cloaca, as described before, receives its principal blood supply from the posterior continuations of the internal iliac arteries.

From the above facts it is concluded that the ovary receives its blood supply mainly

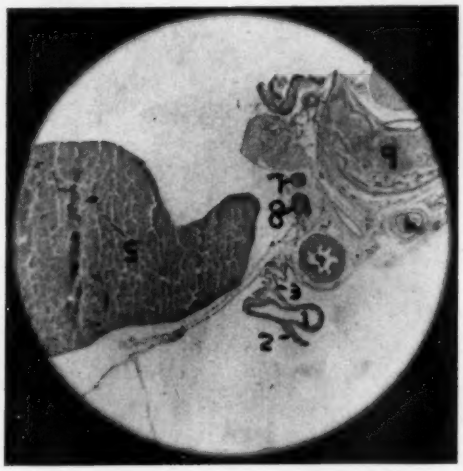

Fig. 5. Cross section of an 11-day-old Black Giant chick, with the vertebrae and surrounding structures shown from a posterior view. (1) Oviduct. (2) Ventral ligament of the oviduct. (3) Dorsal ligament of the oviduct. (4) Left ureter. (5) Left kidney. (6) Aorta. (7) Spinal nerve. (8) Left sympathetic trunk. (9) Ventral portion of vertebrae.

through the ovarian branch from the aorta, with a secondary branch or branches coming from the left renal.

The oviduct receives its blood supply from three main sources: the anterior part from the left renal or left femoral, the middle portion from the left sciatic and the posterior portion from the left internal iliac.

SUMMARY

1) The innervation of the female genitalia in the common fowl is derived from the sympathetic system.

2) The ovary is supplied by a large ovarian plexus located just above the ovary.

3) The oviduct is supplied by fibers which emanate from the ovarian, left renal, abdominal aortic, sciatic and pelvic plexuses. It also receives fibers directly from the posterior continuation of the sympathetic trunk.

4) The cloaca receives its nerve supply from the pelvic plexus and from the sacral and coccygeal nerves.

5) The following chief branches were found originating from the abdominal aorta of the cockerel:

a) Intercostal
b) Coeliac
c) Anterior mesenteric
d) Spermatic
e) Renal
f) Femoral
g) Sciatic
h) Posterior mesenteric
i) Internal iliac
j) Lumbar

6) The ovary of the hen derives its blood supply from the ovarian artery and a branch from the left renal.

7) The oviduct of the hen receives its blood supply from the left renal or left femoral, the left sciatic and the left internal iliac.

8) The cloaca receives its blood supply principally from the internal iliac arteries.

Carriers of Hexamita Meleagridis*

W. R. HINSHAW, D.V.M., Ph.D., and E. McNEIL, Ph.D.
Davis, Calif.

NATURAL OR SO-CALLED normal carriers are recognized as the most important means of transmission and perpetuation of infectious diseases. Where individual sanitation is impossible, as is usually the case in rearing turkeys, chickens and other animals, the carrier problem is serious. Where there are several age groups (including the parent stock) on a small area of ground, the problem is particularly acute.

In our early studies on infectious catarrhal enteritis of turkeys[1] we recognized that the survivors of the infection were the principal source of the causative parasite (*Hexamita meleagridis*). Studies have since been made to determine the incidence of *H. meleagridis* in adult turkeys which have survived acute outbreaks and in other birds and animals which were killed on or near ranches where acute outbreaks have occurred. These, and the results of some cross-infection experiments, are reported in this paper.

MATERIALS AND METHODS

Domestic birds were examined either on ranches or at autopsy. Wild birds and other animals were killed for examination in the vicinity of infected premises.

In field surveys of sexually immature birds we have found the bursa of Fabricius a convenient source of material for examination. In turkeys the parasites, if present at all, will be found in the bursa and, unless contaminated with rectal contents, will usually be free from other protozoa.

The method of collecting bursal scrapings consists of inserting a probe, toothpick, applicator stick or a small curette into the bursa with care to avoid fecal contamination. A small scraper made from wire or glass of suitable diameter has been used with success for this purpose as has also a facial curette which can be purchased at any drug store for a few cents. The epithelium, which in this organ desquamates easily, is gently scraped and the specimen thus obtained is suspended on a glass slide in a drop of physiologic saline or Locke's solution. A cover slip is placed over the drop and the specimen is examined with the aid of the high dry objective of a microscope. The flagellates are easily recognized by their fleet-forward type of movement and by their tendency to cling to bits of tissue by their flagella, or by their sucking discs. They can be differentiated from Trichomonas by the absence of an undulating membrane. The average size of *H. meleagridis* is 9μ x 3μ.

The examination of live, sexually mature birds was made by obtaining rectal scrapings, with the aid of a curette or a similar instrument. In birds killed for autopsy the parasites may be found most frequently in the scrapings made from, or in the region of, the cecal tonsils* located about 2 to 4 mm. from the cecal-rectal opening. Scrapings from this area and from the bursa or rectum, or both, were routinely made in the case of all specimens necropsied.

It is essential to use either physiologic saline or Locke's solution as a diluent. Warming the slide slightly aids in increasing the motility of the organism. Dr. James Harr of the California state department of agriculture reports that immersing a tied-off section of the unopened intestine, from birds that have been dead for a few hours, in warm saline solution before making scrapings will increase the chances of finding the Hexamita. A common error is to use too much material. If the organisms are present at all, they can best be seen in a thinly prepared smear. Inasmuch as the examination of fixed and stained smears did not have any advantage over the examination of fresh scrap-

*The specific name meleagridis has been suggested for this parasite by McNeil, Hinshaw and Kofoid.[2]

From the Division of Veterinary Science, University of California. Presented before the Section on Poultry at the seventy-seventh annual meeting of the American Veterinary Medical Association, Washington, D. C., August 26-30, 1940.

[1]Hinshaw, W. R., McNeil, E., and Kofoid, C. A.: The relationship of Hexamita sp. to an enteritis of turkey poults. Cornell Vet., xxviii (1938), pp. 281-293.

[2]McNeil, E., Hinshaw, W. R., and Kofoid, C. A.: *Hexamita meleagridis sp. nov.* from the turkey. Am. J. Hyg., xxxiv (1941), Sec. C, pp. 71-82.

*In previous papers these glandular bodies have been referred to as ileocecal glands. We are indebted to Dr. L. Rosenberg, Division of Zoölogy, University of California, for calling our attention to the correct nomenclature. Dr. Rosenberg (unpublished data) has shown that the ceca of turkeys empty directly into the rectum; hence, the term ileorectal opening is the correct terminology. Muthmann, according to Calhoun,[3] first used the term cecal tonsil in 1913.

[3]Calhoun, M. L.: The microscopic anatomy of the digestive tract of *Gallus domesticus*. Iowa State J. Sci., vii (1933), pp. 261-382.

TABLE I—Incidence of Hexamita in Birds and Other Animals

Host	No. Examined	No. Ranches	Positive for Hexamita	
			No.	Per Cent
Adult turkeys				
Rectal	79	5	13	16.5
Cecal tonsil	74	4	24	32.4
Chickens*	29	3	0	0
Ducks	3	2	0	0
Pigeons†	32	7	8	25.0
Guinea hens	4	2	0	0
Pea hen	1	1	0	0
Quail	41	3	18	43.9
Chukars	14	1	1	7.1
Pheasants	6	1	0	0
Sage hen	1	1	0	0
Jungle Fowl	1	1	0	0
Grouse	1	1	0	0
Blackbirds (Brewer's)	18	3	0	0
English sparrows	8	4	0	0
Linnets	3	1	0	0
Butcher birds	2	1	0	0
Towhees (Anthony's)	2	1	0	0
Hawks (Red-tailed)	1	1	0	0
Owls (Screech)	1	1	0	0
Kingbird (Arkansas)	1	1	0	0
Crows	1	1	0	0
Lizards	1	1	0	0
Toads	2	1	0	0

*In addition to many hundreds examined by state diagnostic laboratories and at the Pomona demonstration plant.

†Eight pigeons were positive for *H. columbae*. (See text.)

ings suspended in saline, it was given up after a few trials.

EXAMINATIONS OF CARRIERS

Three groups of birds (domesticated, game and wild) are included in these studies; also the results of examination of one alligator lizard and two toads, all killed in brooding yards where an acute outbreak was in progress. A summary of the results of examinations is given in table I.

I. DOMESTICATED FOWLS

Turkeys.—A total of 79 live breeders, 1 year of age or older, were examined on five ranches. All of these birds were survivors of outbreaks of infectious catarrhal enteritis which had been diagnosed by us, and the flocks kept under our observation throughout the season. Of the 79, 41 were females, 38 males. Thirteen (16.5 per cent) of the birds were found by rectal examination to be positive for Hexamita. Of these 13, six were females, five males. Taking the sexes separately, there was a 14.6 per cent incidence in the females and a 13.2 per cent incidence in the males. In ten of the birds there were only a few Hexamita. In the other three (two males and a female) Hexamita was abundant. These three birds were on three separate ranches and it is significant that they were definitely subnormal. One of these was used for transmission studies reported elsewhere in this paper.

On one ranch we examined twelve birds (six males and six females) not included in the above data and found them all negative. This flock had suffered no loss from infectious catarrhal enteritis as poults; Hexamita had never been found in them. This is particularly encouraging, since in years past the same grower had suffered as high as 80 per cent mortality from this disease. The only change he made was to practice rigid sanitation and complete segregation of his breeding stock and poults. The poults were reared in brooders, with sand for litter, and outdoor runs which were not screened.

Seventy-four adults from four infected ranches were examined at autopsy. Of these, 24 (32.4 per cent) were found positive for Hexamita by examination of the cecal tonsil. This is, of course, more nearly the correct figure for incidence of Hexamita in adult birds. The percentage of incidence is, therefore, high enough in breeders to account for the transmission of the disease to young birds.

Chickens.—The chicken is the domestic bird most often found in contact with turkeys. We examined 29 chickens on three ranches where the poults were suffering from an outbreak of this disease. All were free from Hexamita. A more significant fact is that the state poultry diagnostic laboratories examine hundreds of chickens each year and have never reported it. (See "Cross-Infection Experiments.")

Thirty-two pigeons from seven sources were examined and eight (25 per cent) were positive for *H. columbae*. This species is not transmissible to turkeys, although it produces a catarrhal enteritis in pigeons (Nöller and Buttgereit[4]). Three ducks, four guinea fowls and one pea hen were examined; all were negative.

II. GAME BIRDS

Forty-one quail from three sources were examined and 18 (43.9 per cent) found positive for Hexamita. One of these positive sources was a game farm, the other was a bird brought in from the wild. We observed two outbreaks of infectious catarrhal enteritis in poults where there was good field evidence that quail had been the carriers.

Hexamita was found in one of 14 chukars examined. These chukars were on the same game farm where Hexamita was found in quail. Since many chukars are now being liberated from game farms, they may prove to be a carrier of infectious catarrhal enteritis. Six Chinese ring-necked pheasants were examined; all were negative for Hexamita. One each of sage hen, jungle fowl and grouse were also negative for Hexamita.

III. WILD BIRDS

Brewer's blackbird, English sparrows and linnets are all often found in turkey yards. Eighteen black birds, eight sparrows and three linnets were examined. Hexamita was not found in any of these species. It is possible that they may occasionally act as a mechanical carrier, but they usually are found in yards at a time of the year when weather conditions are dry and when the probability of their carrying material on their feet would be minimized. Two each of butcher birds, and Anthony towhees, and one each of red-tailed hawks, screech owls, crows and Arkansas kingbirds also were examined and found negative. It should be remembered that all of these birds were killed on or near premises where *H. meleagridis* was known to be present.

IV. MISCELLANEOUS EXAMINATIONS

Lizards and Toads.—One alligator lizard and two toads caught in a brooder yard where an acute outbreak was in progress were negative for Hexamita.

Flies.—We have not been able to incriminate flies as carriers, either by experimental or by field studies. In none of our experiments on transmission have we made any attempt to fly-proof the brooders. In these experiments the control pens remained free from Hexamita in spite of the fact that flies were abundant. For example, in one experiment six wire brooders (sunshine type) were placed about 4 ft. apart, out of doors. Three of these were used for infected poults and three for controls (uninfected). The infected groups were placed so that the wind blew from these toward the control groups. Flies frequented both groups, and the fact that a few of the poults were found infected with tapeworms (*Choanotaenia infundibulum*) upon autopsy is evidence that flies were being eaten by the poults. Both groups were kept under observation for twelve weeks and at the end of this time the controls were still free from Hexamita.

In our field studies microscopic examinations of large numbers of poults reared in infected areas showed that it is possible to prevent transmission by strict sanitation without fly-proofing the brooding equipment.

Microscopic examination of flies caught in infected brooders has failed to reveal any Hexamita. When examining flies it is important to remember that they are often infected with a flagellate of their own (Herpetomonas) which must be differentiated from Hexamita. It is recognized that flies may, under certain conditions, act as mechanical carriers of Hexamita, but the extremely low rainfall and low humidity during the brooding season in many western areas minimizes this type of transmission, because of the short time that the para-

[4]Nöller, W., and Buttgereit, F.: Über eines neues parasitischen Protozoons der Haustaube (*Octomitus columbae nov. spec.*). Cent. f. Bakt., Abt. I, Orig., lxxv (1923), pp. 239-240.

sites will remain alive after exposure to the dry atmosphere.

TRANSMISSION FROM ADULT TURKEYS

Field evidence has consistently indicated that there is a build-up of infection from the adult carrier through early groups of poults until there is a dosage heavy enough to start an acute outbreak. One experiment has been completed to confirm field evidence that this is true.

A subnormal yearling male was brought in from an infected ranch and a physiologic saline suspension made of the rectal contents. Although Hexamita was numerous, it was not as abundant as in a typical picture of the disease. Six poults were each given 0.5 cc. rectally and 0.5 cc. orally of this inoculum. At the end of nine days one of the poults had shown no gain in weight. It was sacrificed and autopsy showed Hexamita to be abundant in the jejunum, ileum and bursa. This organism also was present in the bursa of the other five poults, but was not abundant. None of these died.

Material from the intestine of the poult which had been killed was fed to two uninfected poults (2 weeks old). Hexamita was approximately three times more abundant in this suspension than in that from the adult birds. One of the two birds died of typical infectious catarrhal enteritis. Hexamita was even more abundant in this bird.

Material from this second serial passage was given to five other poults. As soon as symptoms appeared, 13 more birds were put in the pen. All five of the inoculated birds and twelve of the contacts died. Thus, in this experiment it took two serial passages in young poults to increase the number of organisms to a dosage sufficient to produce typical acute cases of the disease. Beginning with the third series, it was possible to keep the acute disease progressing in the pen by the introduction of susceptible poults as contacts.

At the same time that the above experiment was being conducted, a parallel one, in which inoculum from an acute field outbreak in poults was used, was conducted. Poults from the same hatch and from the same parent stock were used for both. Six poults were inoculated in the same manner as in the adult transmission experiment and 21 poults were placed in the pen as contacts. All of the six inoculated poults and 20 of the 21 contact poults died from this single-series inoculation. The number of Hexamita in the inoculum used for this transmission was comparable with the number used in the third serial transmission in the other experiment.

CROSS-INFECTION EXPERIMENTS

Cross-infection experiments have been described in detail in another paper.[2] Therefore, only a brief summary will be included here.

The species of Hexamita found in pigeons is not transmissible to turkeys. Although *H. meleagridis* can be established in baby chicks, it does not spread to other chicks in the same brooder and lasts only a few weeks. No symptoms or stunting have been noted in chicks, even when excessive dosages have been given. *H. meleagridis* persists in ducks for at least two months, but no symptoms have been noted. A species of Hexamita found in quail and chukars is transmissible to turkey poults. *H. meleagridis* also is transmissible to quail.

DISCUSSION

It is a recognized fact among turkey growers that if there are several age groups on a ranch, the earlier hatched birds may escape serious losses from infectious catarrhal enteritis, while the later hatched lots suffer heavy mortality. The experimental build-up of dosage by serial passage of an inoculum secured from an adult carrier turkey to a stage where the acute disease could be produced at will has helped to explain this field picture. All of our experimental work to date has indicated that this effect is due to increase in dosage rather than to increase in virulence.

Another possible factor in the production of the acute disease is the increase in the number of parasites in subnormal breeders so often seen late in the season. The male

used for the carrier-transmission experiment reported herein is an illustration of this factor. This bird had been culled from the breeding flock because of its poor condition. The number of the parasites found on autopsy was smaller than that found in poults suffering from the acute disease, but larger than in normal adult carriers. With these subnormal individuals on the ranch, the chances are, of course, greater for transmission of dosages sufficient to start an acute outbreak and may explain some outbreaks. The habit of turkey growers to keep a few subnormal birds on the premises after the main flock has been sold has helped to explain outbreaks in poults hatched after the disposal of the main breeding flock, and where earlier groups were not present.

There are almost always adult turkeys on breeding ranches when the first group of poults is received. This first group usually receives better care than the others because there is less work for the grower to do than later in the season. The poults may, therefore, receive only a few Hexamita and reach an age-resistance stage without showing symptoms. The following groups, unless great precautions in isolation are taken, will receive a greater dose than the first. There are several reasons for this: (1) There are probably more organisms present in the poults than in the adult birds (age factor); (2) the grower is increasingly busy and is often tempted to treat age groups as one unit; (3) he has sold his breeders and forgets that the older age group of poults is a potential danger; (4) in some cases the older poults fly out or burrow under fences and get in with the younger ones.

Preliminary studies and field observations also indicate that the better physical condition often noted in the early broods and a possible acquired immunity may be factors in preventing a dosage build-up in early hatched groups.

Subnormal poults in a brood also may help to step up dosage. We have observed both in the field and in our experimental studies that this is true. The poult sacrificed for inoculation for the second serial transfer in the adult-transmission experiment was such an example. The bird was underweight when infected and showed no gain in weight for nine days after being given the original dose. Autopsy showed it to have only a remnant of one lung and an abnormal kidney—abnormalities which may have allowed the infection to progress more rapidly than in the other poults and produce organisms in great enough abundance to cause acute symptoms if picked up by susceptible poults. In the field subnormal poults have been observed to have large numbers of the organism which may act as foci for transmission. Other diseases, such as paratyphoid and pullorum disease, also may predispose poults to this disease.

SUMMARY

1) *Hexamita meleagridis*, the cause of infectious catarrhal enteritis, was found in the intestine of 16.5 per cent of live adult turkeys by rectal examination, and in 32.4 per cent at autopsy.

2) In sexually immature birds the bursa of Fabricius was found to be a convenient source of material.

3) The incidence of infection of *H. meleagridis* in adult turkeys is high enough to prove that the breeding stock is the most important source of infection.

4) The following domestic birds were examined and found negative for *H. meleagridis*: chickens, pigeons, ducks, guinea hens and pea fowls. *H. columbae* was found in 8 (25 per cent) of 32 pigeons examined. This species was not transmissible to poults.

5) The following game birds were examined: quail, chukars, pheasants, sage hens, jungle fowls and grouse. A species of Hexamita transmissible to poults was found in quail and chukars. The others were negative.

6) The following wild birds were examined and found negative for Hexamita: blackbirds, English sparrows, linnets, butcher birds, towhees, hawk (1), owl (1), kingbird (1) and crow (1).

7) Two toads and one alligator lizard

were negative for Hexamita.

8) It has not been possible to incriminate the fly as a carrier.

9) The acute type of infectious catarrhal enteritis was produced in poults by oral and rectal injections of saline suspensions of *H. meleagridis* obtained from an adult carrier. This was accomplished by serial transfers in poults until heavy losses occurred in the group receiving the third serial injection.

10) Cross-infection studies indicate that quail, chukars and possibly ducks may act as carriers of *H. meleagridis*.

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ADDENDUM

Since this paper was submitted for publication the writers have obtained additional data on artificial infection of chickens with *H. meleagridis* (McNeil and Hinshaw[5]). In these studies the parasite persisted in the bursa of Fabricius and cecal tonsils for at least 22 weeks when the initial inoculation was made in chicks under 1 week of age.

No symptoms of disease were ever produced. Because of these findings chickens also must be considered potential carriers, even though the parasite has not been found in farm flocks under natural conditions.

[5]McNeil, E., and Hinshaw, W. R.: Experimental infection of chicks with *Hexamita meleagridis*. Cornell Vet., xxxi (1941), pp. 345-350.